

THESIS

DEVELOPMENT OF JIAOGULAN TEA (*Gynostemma pentaphyllum*)

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THESIS

DEVELOPMENT OF JIAOGULAN TEA (*Gynostemma pentaphyllum*)

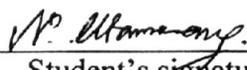
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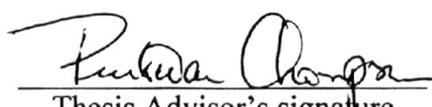
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Jiaogulan (*Gynostemma pentaphyllum*) is a herbal medicine know to decrease blood lipid level and been used as an anticancer agent. It is originally grown in China but can be grown in the North of Thailand. Aim of this study was to develop Jiaogulan tea for consumers who wanted to maintain their cholesterol and triglyceride. This research was divided into four phases. The first phase, the study of active ingredients of Jiaogulan indicated that the Jiaogulan leaves contained more saponin (204.67 mg/g) and antioxidant activity (23.04 mg Trolox/g) than the stem. So, Jiaogulan leaf was selected to be raw material of the product. The second phase, the raw material was prepared by two drying processes; hot air drying (40, 50, 60°C) and vacuum-microwave drying (1600, 2400, 3200 watt). Results showed that total saponin contents were not significant difference. In contrast, the antioxidants were significantly different ($p \leq 0.05$), which vacuum-microwave dried Jiaogulan contained 37.98-56.72 mg Trolox /g while hot air drying reduced the antioxidant properties to 4.48-7.71 mg Trolox/g. Thus, the optimum process was vacuum-microwave drying at 2400 watt for 25 min. The third phase was identification of Jiaogulan water extract. The GC-MS chromatogram showed 2 peaks of gypenosides and their mass spectrum matched with ginsenoside Rb1 and Rg1. Furthermore, the Jiaogulan water extract was studied on the effect on serum lipid in hypercholesterolemia rats. Ten rats were fed with basal diet for 10 days, and then fed with high cholesterol diet (1 g % w/w cholesterol) for 14 days. The cholesterol levels were significantly increased just period to feed 3 g % Jiaogulan extract for 2 weeks. The results showed that Jiaogulan extract could significantly reduce triacylglycerol levels and lower cholesterol level. The final phase was the development of herbal beverage from dried Jiaogulan. A consumer survey (n=416) results indicated that the target consumer were those age over 55 years old and the product profile as dry leave powder in a tea bag with natural flavor was preferred. Therefore, the further study was to verify the developmental process of Jiaogulan tea infusion. The study found that infusion temperature was at 90°C for 10 min in extracting the maximum saponin and having highest antioxidant property. The optimum Jiaogulan tea consisted of 292 mg of saponin with 100 ml of water. The formula was confirmed by the consumer acceptance test (n=200). The hedonic ratings were moderately like (6.42-7.17). Ninety-two percent of respondents accepted this tea and the McNemar test indicated that the health benefit information could change buying decision of consumers. The final product qualities were correlated with the Thai standard of Jiaogulan herb.


Student's signature

 12 / 7 / 2006
Thesis Advisor's signature

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DEVELOPMENT OF JIAOGULAN TEA

(*Gynostemma pentaphyllum*)

INTRODUCTION

Functional foods are popular in many countries such as the United States, Japan, Europe and also recently Thailand. The global functional foods market is estimated to be \$ 47.6 billion in 2001 and has grown up very fast from around \$ 30 billion in 1995. In the U.S. market, beverages are projected to remain the largest segment, followed by breads and grains (Sloan, 2002). The nutraceutical beverage market, including tea, sport beverage, energy beverage, new age beverage, fortified juice and milk and isotonic, has been growing rapidly over the last several years (Wright, 2005). One of the most important functions which consumer need is helping to maintain cholesterol levels that is related to the risk of heart disease (Sloan, 2002). Cholesterol is a lipid steroid molecule in blood which is necessary for human life and the normal blood should contain a total cholesterol level < 200 mg/dl, low density lipoprotein (LDL) cholesterol < 130 mg/dl, and total triglyceride < 135 mg/dl. If the cholesterol level is higher than normal (hypercholesterolemia), it will be the main cause of atherosclerosis and coronary heart disease (Goldberg, 1994; Gibson and Williams, 2000). In Thailand, there are many Thai people who have cholesterol problems. Utma-ang *et al.* (2003) survey with 441 Thai patients, they need to have functional food to maintain their cholesterol more than drug, which is so expensive. Therefore, functional food should be the alternative way to solve this problem.

Jiaogulan, *Gynostemma pentaphyllum*, is a Chinese medicinal herb which is called “Panjakan” in Thai and “ Amachazuru” in Japanese (Blumert and Liu, 2003). Jiaogulan is a perennial liana in the *Cucurbitaceae* family. This plant grows around the high land in Northern Thailand; Chiangmai and Chiangrai. The important compositions are saponin glycosides and antioxidant (The Medicinal Plant Research Institute, 2005). Phytochemical studies of this plant have identified about 90 dammarane- type glycosides (called gypenoside) closely related to ginseng saponins

(Cui *et al.*, 1999). Comparing to ginseng, Jiaogulan is cheaper and easily available. Pharmacological studies of Jiaogulan and the isolated saponin have shown a variety of interesting activities such as cholesterol-lowering, immunopotentiating, anticancer, antioxidants and the others (Blumert and Liu, 2003). The State Food and Drug Administration, People's Republic of China allowed Jiaogulan to be used in functional food, as well as Taiwan that it can be used as food ingredient (Chau and Wu, 2006). Therefore, Jiaogulan has highly potentiality to be the raw material of functional food and nutraceutical for lowering cholesterol and antioxidant effect.

Aims of this research are to study the active ingredients and to develop herbal beverage from Jiaogulan for consumers' age over 35 who want to maintain their cholesterol and triglyceride. The specific objectives of this study are as following:

1. To examine the active ingredient in fresh Jiaogulan as raw material
2. To select optimum drying process of Jiaogulan in order to maintain the active ingredient
3. To study the effective of Jiaogulan extract on rat serum lipid
4. To develop the Jiaogulan tea

LITERATURE REVIEWS

1. Jiaogulan

1.1 Morphology and Cultivation

Jiaogulan (jee-ow-goo-lahn), the scientific name is *Gynostemma pentaphyllum* (GP) in family *Cucurbitaceae*, is a perennial liana which stems grow like vines and leaves has five oval-shaped with saw tooth edges and white hairs (Figure 1). Jiaogulan can be cultivated from the seed or from plant cutting. Jiaogulan requires a rich well drained humus rich soil in a warm sheltered area with partial shade. It can not grow well in cold climates where temperature drops down to freezing. Jiaogulan could harvest in 4-5 months (Takemoto, 1984). Jiaogulan grows abundantly in Southern China, Japan, and Korea. In Thailand, it grows wild in the Northern, which is native strain. Another cultivar came from China last 10 years ago, and Jiaogulan can grow easy with good quality in Thailand. Now, there are a lot of Jiaogulan in the Northern of Thailand such as in Chiangmai, Chiangrai and Maehongsone. The Royal Project promotes Jiaogulan to the hill tribes in Chiangmai and develops to the agronomic production.

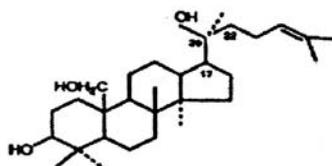


Figure 1 Jiaogulan plant (*Gynostemma pentaphyllum*).

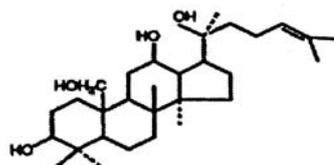
Source: Blumert and Liu (2003)

1.2 Phytochemical Composition

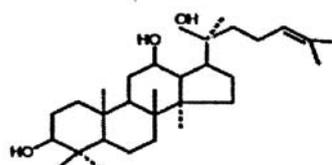
Phytochemical studies of Jiaogulan contain saponins, flavones, polysaccharides, amino acids, vitamins and minerals, including many essential trace elements. Among these, the saponins are the most important of effective components. Kawahara *et al.* (1989) screened wild plants of GP for ginseng saponins, and isolated new compounds called 6''-malonylginsenosides-Rb₁, -Rd and 6'-malonylgypenoside V from the leaves of cultivated plants. Hu *et al.* (1997) have identified dammarane-type glycosides, called gypenosides, which closely related to the ginseng saponins. Indeed, gypenosides III, IV, VIII, XII and malonyl gypenosides III and VIII are identical to ginsenosides Rb₁, Rb₂, Rd, F₂ and malonyl ginsenosides Rb₁ and Rd. Cui *et al.* (1999) identified four main dammarane-type aglycones of gypenosides from GP which are difference from *Panax* spp., are 20(S)-dammar-24-ene-3 β , 19, 20 triol ; 20(S)-dammar-24-ene-3 β , 12 β , 19, 20 tetrol; 20(S)-protopanaxadiol and 20(S)-dammar-24-ene-2 α , 3 β , 12 β , 20 tetrol (Figure 2 (1), (2), (3), (4)). The Medicinal Plant Research Institute (2005) concluded that four similar saponins in Jiaogulan and ginseng are ginsenoside Rb₁ (gypenoside III or gypensaponin C), ginsenoside Rb₃ (gypenoside IV), ginsenoside Rd (gypenoside VIII) and ginsenoside F₃ (gypenoside XII).



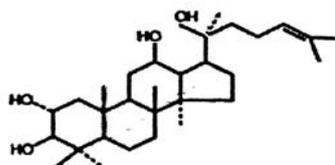
(1) 20(S)-dammar-24-ene-3 β , 19, 20 triol



(2) 20(S)-dammar-24-ene-3 β , 12 β , 19, 20 tetrol



(3) 20(S)-protopanaxadiol



(4) 20(S)-dammar-24-ene-2 α , 3 β , 12 β , 20 tetrol

Figure 2 The structure of dammarane-type aglycones of gypenosides from Jiaogulan.

Source: Cui *et al.* (1999)

1.3 Therapeutic Qualities of Jiaogulan

1.3.1 Antioxidant

The antioxidant effect that Jiaogulan can prevent the free radical damage caused by oxidation of cell membrane lipids and biological molecules. This is closely related to variety of maladies such as cancer, atherosclerosis, diabetes, ischemic lesions, liver disease, arthritis, inflammation and the regressive changes of the aging process. Vascular cells are exposed to oxidation during inflammation, endotoxic shock, possibly hypertension. There are 4 principle cells involved in atherosclerosis : endothelium, smooth muscle, platelet, monocyte/macrophages. Oxidant injury of the endothelium is considered an early event in the development of atherosclerosis (Blumert and Liu, 2003). Lin and Lau (1993) and Lin *et al.* (1993) studied the antioxidant effect of gypenosides using various models of oxidative stress in phagocytes, liver microsomes and vascular endothelial cells. Results showed that gypenosides decreased superoxide anions and hydrogen peroxide content in human neutrophils and diminished chemiluminescent oxidative burst triggered by zymosan in human monocytes and murine macrophages. It was also found that gypenosides protected biomembranes from oxidative injury by reversing the decreased membrane fluidity of liver microsomes and mitochondria, increasing mitochondrial enzyme activity in vascular endothelial cells and decreasing intracellular lactate dehydrogenase leakage from these cells. The clinical studies by Liu (1994) and Lu (1998) showed that gypenosides can induce endogenous production of superoxide dismutase (SOD), which can scavenge free radicals, hence preventing the lipid peroxidation of cell membrane and lowering the production of MDA. In another aspect of this study, hydrogen peroxide was used as a model to induce lipid peroxidation and biomembrane damage, including a decrease of enzyme activity and a disruption of integrity vascular epithelial cell. Hydrogen peroxide at 62.5 μM and 125 μM caused a decrease of cell viability by 62.3% and 94.7% respectively. Pre-incubation of GP at doses of 25-150 μM peroxide. Lactate dehydrogenase (LDH) is an intracellular enzyme which leaks into the culture medium when cell membranes are damaged. Exposure of cells to the identical concentrations of peroxide listed

above caused a significant increase of LDH leakage, by 223.4% and 419.8% over control. Pre-incubation for 6 hours with 25-150 W. on GP resulted in a dose dependent decline of LDH release from peroxide treating cells. These extensive studies demonstrated the antioxidant effect of GP on vascular endothelial cells and monocyte/ macrophages, suggesting that GP may contribute to the prevention and treatment of atherosclerosis and other cardiovascular diseases (Lu, 1998).

1.3.2 Lowering Cholesterol.

Jiaogulan has been shown to reduce the serum level of triglycerides, lipid peroxide, total cholesterol, phospholipids, and glutamic pyruvic transaminase in animal studies which has been treated by crude saponin isolated from GP (Kimura, 1983). La Cour *et al.* (1995) evaluated the ability of a decoction of 3 species; *Crataegus cuneata*, *Nelumbo nucifera* and *Gynostemma pentaphyllum*; to lower cholesterol and triglycerides in a short-term experiment with rats and quails. Animals were fed high lipid diets and the herbal decoction for one week. The decoction of the plants was prepared from dried material by extracting with water, boiling for one hour, and storing under refrigeration. In rats, an average of 20 replications showed that the effect of GP was not linear, but rather showed an optimum dose of 2 g/kg per day, for decreasing both cholesterol and triglycerides levels in animal blood. Qi *et al* (1996) investigated the influence of gypenosides on serum lipoprotein and atherosclerosis in hyperlipidaemia animals. Results indicated that gypenosides can suppress the rise of serum cholesterol and triglycerides in hyperlipidaemia mice and lower the content of cholesterol triglycerides and LDL in hyperlipidaemia quails. There were the cholesterol lowering compounds which can reduce LDL but increase HDL, including; gypenoside IX, gynosaponin A, B, E, F, G, J, K, M, N, O, progynosaponin A2, A-AH and O (Tekemoto *et al.*, 1983; Takemoto, 1984 a, 1984 b, 1984 c, Takemoto *et al.*, 1984; Wang *et al.*, 1995)

Many clinical studies on the therapeutic effectiveness of Jiaogulan on hyperlipemia were summarized by Blumert and Liu (2003) that the effectiveness rate ranging between 67-93% on more than 980 patients with

hyperlipemia. Yu (1993) carried out testing in 30 human cases with hyperproteinemia; the total effectiveness rate of GP to lower high blood lipoprotein was 86.7%. A great number of tests in human testing have confirmed Jiaogulan's ability to decrease the levels of serum cholesterol, triglycerides and LDL, while increasing serum HDL level, thus decreasing the LDL/HDL ratio.

1.3.3 Adaptogenic Effect

Adaptogens play the important part by helping the body to adapt to the stress and maintain its homeostasis. Gypenosides have been proved in animals and human testing to be a highly effective adaptogenic tonic. Jiaogulan exerts a bi-phasic action on the central nervous system. It calms the irritation and excitement or depressive state of the nerves, through adjusting the balance of the central nervous system (including the brain, sympathetic and parasympathetic nerve systems) and toning the endocrine system. Gypenosides maintained and normalized the functional equilibrium between the organs of the body, then bolstered the stability of the organism as a whole (Blurmet and Liu, 2003). Zhou (1990) showed the adaptogenic effects of Jiaogulan on mice. The report showed that gypenosides enhanced the endurance and resistance to hypoxia (lack of oxygen), overloaded exercise, electric stimulus and high temperature. Clinical testing, gypenosides were also effective in treating insomnia due to anxiety and irritation. The study in 112 cases showed that gypenosides could improve sleep with the effectiveness rate of 89-95% (Liu, 1993).

1.3.4 Enhance Cardiovascular Function

Jiaogulan improved the efficiency in the pumping action of the heart. Chen (1990) investigated the effects of gypenosides on the heart function and hemodynamics in anesthetized open-chest dogs, and found that gypenosides, 5-10 mg/kg, significantly lowered either systolic, diastolic pressure or mean arterial blood pressure. Total peripheral vessel resistance, brain vessel resistance, and coronary resistance were markedly decreased. The coronary flow rate was raised while the heart rate was decreased. The myocardial oxygen consumption was low after the

administration of gypenosides. This could be explained by the decreasing of the cardiac tension-time index and the slow heart rate, in the face of effective myocardial contractility and heart pump function. In this study, a comparison of the effectiveness was made between gypenosides and ginsenosides from *Panax ginseng*. Gypenosides were shown to be more potent. Circosta *et al.* (2005) presented that the cardiovascular activity of the aqueous extract of *G. pentaphyllum* Makino leaves were investigated in the anaesthetized guinea-pigs and had been compared with two of its isolated gypenosides (III, VIII) and with verapamil, a well-known Ca-antagonistic drug. The results showed that the intravenous administration of decoction of *G. pentaphyllum* (2.5, 5 and 10 mg/kg) produced a protective effect against induced coronary spasm, arrhythmias and pressor response. The extract also increased the dose of ouabain required to cause ventricular tachyarrhythmias and lethality. Doppler echocardiography was carried out in 30 healthy persons and 220 athletes receiving gypenosides (Zhou, 1993). Positive results were shown in all after oral administration of a single dose of gypenosides combined with other Chinese medicinal herbs for 30 minutes subjects. The results were the increased stroke volume and cardiac output, the decreased left ventricular end-systolic diameter and the increased left ventricular end-diastolic diameter. However, the heart rate and arterial blood pressure did not change.

1.3.5 Lowering Blood Pressure in Patients with Hypertension

If the arterial blood pressure at the time of contraction (systole) of the heart exceeds 140 mm Hg, and during expansion (diastole) is over 90 mm Hg, then hypertension is diagnosed. Hypertension will cause serious complications of the brain, heart and kidneys. On the other hand, if systolic blood pressure is lower than 80 mm Hg, and diastolic pressure is lower than 60 mm Hg, then hypotension is diagnosed. Hypotension will cause such bad effects as cerebral ischemia, collapsed. Gypenosides from *Jiaogulan* are similar to the ginsenosides of *Panax ginseng*, in that they have lower hypertension and raise hypotension, keeping the blood pressure at a normal range (Blurmert and Liu, 2003). Tanner *et al.* (1999) demonstrated that GP stimulates the release of nitric oxide, a substance that causes blood vessels in the body

to relax. The study concluded that this maybe one mechanism by which gypenoside reduces blood pressure. Lu (1996) studied the anti-hypertension effect of gypenosides in a double blind study. In this study, 223 patients with essential hypertension (grade II) were randomly divided into 3 groups: Group1 consisted of 78 patients; 20 mg gypenosides tablets were administered orally, twice a day for three months. Group 2 consisted of 82 patients; 20 mg ginsenosides tablets were administered orally, twice a day for 3 months. Group 3, 65 patients; 1.25 mg Indapamide (an effect anti-hypertensive medicine, an alpha receptor inhibitor) were given orally, twice a day for three months. The results showed that the effectiveness rates were 82%, 46% and 93% for gypenosides, ginseng and Indapamide, respectively.

1.3.6 Prevent Heart Attack and Stroke

Platelet aggregation is the basis for formation of blood clots in the blood vessels of living animals or humans (thrombosis) and accumulation of plaque in the arteries (atherosclerosis). Jiaogulan has the ability to inhibit the aggregation of blood platelets. Hence, these results suggest that GP ability to assist in preventing cerebral thrombosis from stroke and preventing coronary thrombosis from heart attract (Blumert and Liu, 2003). Tan et al. (1993) observed the antithrombotic effect of water extract of GP both in vitro and in vivo, and found that it could significantly inhibit the platelet aggregation induced by ADP and compound antagonists, accelerate the dis-aggregation and thus, effectively inhibit the experimental thrombosis. Yu (1995) performed a test in 56 healthy persons and 44 patients with cardiovascular diseases. The results showed that a single dose water extract of Jiaogulan (containing gypenosides 30 mg/ml), 1 ml/kg of body weight orally, inhibited platelet aggregation and promoted platelet dis-aggregation, indicating the anti-thrombotic effect of Jiaogulan.

1.3.7 Growth Inhibition of the Cancer Cells

Gypenosides extracted from Jiaogulan have been shown to be effective in controlling various types of cancer cells. Han *et al.* (1995) reported that

GP could inhibit cell cycle of human lung adenocarcinoma cell. Wang *et al.* (1995) performed the experiment on rats. The animals were given a 2% *Gynostemma pentaphyllum* boiled aqueous solution to drink freely for two weeks before administration of a carcinogen. Subsequently, the rats were injected with the carcinogen (MANA) for eighteen weeks and were sacrificed at various time intervals. The results showed that the number of tumors and the incidence of esophageal cancer in the experimental group were lower than those in the control group (MANA only), and the initiation of cancer was delayed for six weeks. This indicated that GP might have some preventive and blocking effects on esophageal cancer in rats. However, the effect of GP on cancer is still in the experimental stage up to date and its effect on human cancer needs further testing.

1.3.8 The Bioactive Study in Thailand

In Thailand, there are some researches about Jiaogulan. Kawpinit (1993) studied the pharmacological activities by ethanolic extraction of *G. pentaphyllum* in rats. The results show that its ethnomedical uses reveals interesting activities such as: CNS depressant, analgesia, anti-ulcer action, hypotension and bradycardia. Poomecome (1999) studied the hypoglycemic activity of extraction from *G. pentaphyllum makino*. The finding indicated that the hypoglycemic activity was due to an ability to stimulate insulin released from the pancreas but not insulin-like activity. In the prolonged treatment (4 weeks), the hypoglycemic activity in streptozotocin-induced diabetic rats is probably due to GP caused a stimulation of surviving pancreatic beta cells to release insulin. Additionally, the cholesterol lowering activity was detected in both normal and streptozotocin-induced diabetic rats. Sinsatienporn *et al.* (2001) conducted to determine the efficacy of GP in 23 patients with hyperlipidemia who received GP powder in capsule at the dosage of 5 g twice a day for 3 months and compared with 21 patients receiving placebo. It is concluded that GP powder in capsules at the dosage of 5 g twice a day for 3 months was safe but not effective in reducing lipids in patients with hyperlipidemia. Finally, Kulwat *et al.*(2001) found that Jiaogulan beverage powder extract maybe able to

induce anticarcinogenic enzyme, but it maybe a cocarcinogen. Therefore, Jiaogulan should be studied more in Thailand.

2. Cholesterol and Lipoprotein

2.1 Cholesterol

Molecular structure of cholesterol is steroid. Its chemical structure and name is $C_{27}H_{45}OH$ and cholest-5-en-3 β -ol, respectively. The main feature shown in Figure 3 which the angular methyl groups at C-10 and C-13, the hydrogen atom at C-18 and the side-chain at C-17 are in β configuration, the hydrogen atoms at C-9 and C-14 are in α configuration and the configuration at C-20 is R (Myant, 1981). Cholesterol is essential to life. It is a precursor of several steroid hormones, including the sex hormones estrogen, progesterone, and testosterone as well as the corticosteroids. The major portion of cholesterol in the body is the precursor of bile acids. Bile acid is necessary for the absorption of fat in intestine. The importance clue to cholesterol metabolism is that most of the bile acids are not lost as feces but are reabsorbed from the lower intestine and recycled to the liver.

Cholesterol is widely distributed in animal and human tissues as cholesteryl esters. The predominant form of cholesterol in the plasma is cholesteryl ester. Three reactions, each catalyzed by a distinct enzyme or class of enzymes, are responsible for the formation of cholesteryl esters in the body. Two of these, acyl-CoA: cholesterol O-acyltransferase (ACAT) and cholesteryl ester hydrolase (Cholesterol esterase) are present in most, if not all, tissues. The third enzyme, lecithin: cholesterol acyltransferase (LCAT), is thought to be active only in the plasma and peripheral lymph. The fatty acyl esters of cholesterol can be hydrolyzed by enzyme to free cholesterol. Enzymes catalyzing the hydrolysis of cholesteryl ester are presented in pancreatic juice and in many animals and human tissues (Myant, 1981).

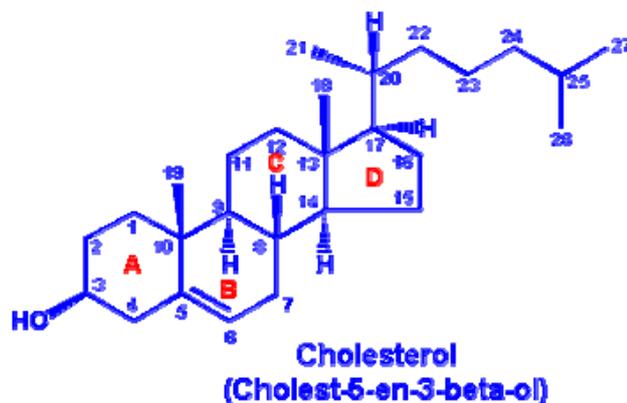


Figure 3 Cholesterol molecule.

Source: Myant (1981)

2.2 Lipoprotein

Lipoproteins represent the functional unit of transport for water –insoluble lipids in the blood. Lipoproteins are water-soluble complexes of high molecular weight composed of lipids (cholesterol, triglycerides, phospholipids) and one or more specific proteins, called apolipoprotein (Assmann, 1982). There are 5 types of lipoproteins; chylomicrons, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low-density lipoprotein (LDL), and high density lipoprotein (HDL). The composition of serum lipoproteins shows in Figure 4 and protein composition of normal human plasma lipoprotein in Table 1. Cholesterol to triglyceride ratio is the highest in LDL. The function of LDL is to pass cholesterol to the liver. There are evidences that high blood level of LDL will cause atherosclerosis.

There is a process of cholesterol recycle in the body. HDL gathers the unesterified (free) cholesterol from peripheral cell. Free cholesterol was esterified by LCAT enzyme in HDL. After that HDL can directly pass cholesterol ester or pass through VLDL and LDL to liver. About half of the cholesterol esters in mature HDL

particles will be delivered to the liver through the HDL receptor. The other 50% are transferred by cholesterol ester transfer protein from HDL to the Apo B-containing lipoproteins VLDL, IDL and LDL and the forward transport of cholesterol recommences. So, the effect of cholesterol transport from cell to liver is very important to cholesterol metabolism and related to the atherosclerosis which can be started to the other chronic disease such as coronary heart disease. The exogenous and endogenous pathways for cholesterol and triglyceride transport are diagrammed (Figure 5). Dietary cholesterol is absorbed through the intestine wall and is packaged along with triglyceride (glycerol ester-linked to three fatty acid chains), in enterocytes as chylomicrons. In the capillaries of fat and muscle tissue the ester bond of triglyceride is cleaved by the enzyme lipoprotein (LP) lipase and the fatty acids are removed and the remaining chylomicrons are called remnants. The further the cleavage the richer the cholesterol. When the cholesterol-rich remnants reach the liver, they bind to the specific receptors and are taken into liver cells. Within liver cells, their cholesterol is either secreted into the intestine as a component of bile salts or is packaged along with triglyceride into VLDL particles and secreted into the circulation, inaugurating the endogenous pathway. Again the triglyceride is removed from VLDL into fat or muscle tissues, leaving cholesterol-rich IDL. Some IDL binds to LDL receptors and is rapidly taken up by liver cells, the remaining stays in the circulation and becomes LDL when triglyceride is further decreased. Most of the LDL binds to LDL receptors on liver or other cells and is removed by circulation (Davidson and Jacobson, 2001).

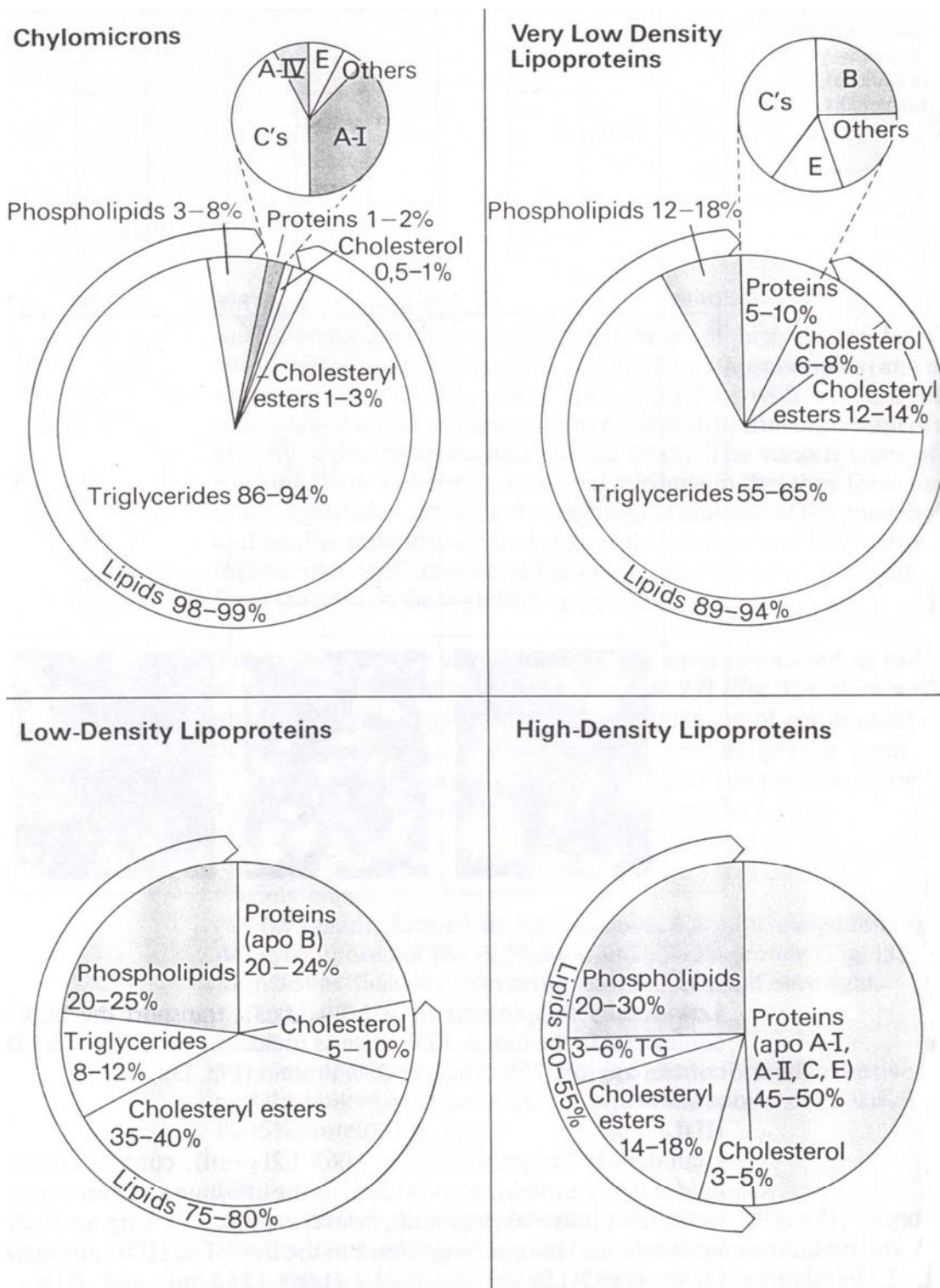


Figure 4 Composition of lipoproteins.

Source: Assmann (1982)

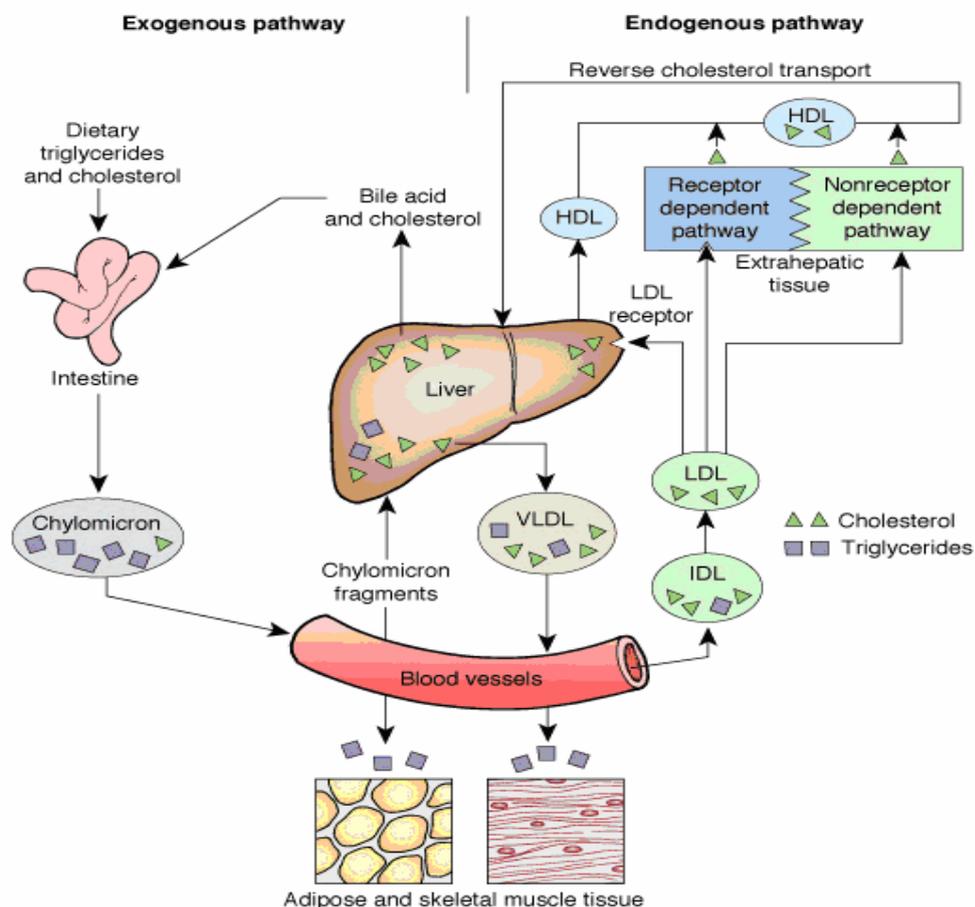


Figure 5 The exogenous and endogenous pathways for lipoprotein and triglyceride.

Source: William and Wilkins (2005)

The National Cholesterol Education Program (NCEP) Panel recommends that people older than age 20 have fasting blood test called a lipoprotein profile every 5 years (Grundy, 2001). The desirable levels of human serum lipid are shown in Table 2. The triad of risk is related to triglyceride, LDL and HDL. The statistic shows that triglyceride is more than 200 mg./dl and LDL/HDL ratio is more than 5 , so the risk of coronary heart disease is 4 times.

Table 1 The lipoprotein properties and protein composition of normal human plasma

Lipoprotein	Density (g/ml)	Diameter (Å)	Molecular weight	Protein constituents	
				Major	Minor
Chylomicrons	<0.95	10^3 - 10^4	10^9 - 10^{10}	ApoB, ApoC	ApoE
VLDL	0.95-1.006	250-750	5×10^6 - 10^7	ApoB, ApoC	ApoE
IDL	1.006-1.019	250	4.5×10^6	ApoB, ApoE	ApoC
LDL	1.019-1.063	200-250	2.0 - 2.5×10^6	ApoB	-
HDL ₂	1.063-1.120	70-120	3.9×10^5	ApoA- I:ApoAII=3.5:1	ApoC
HDL ₃	1.120-1.210	50-120	1.9×10^5	ApoA- I:ApoAII=2:1	ApoC

Source: Myant (1981)

Table 2 The desire value, borderline and high value of human serum lipid

Lipid	Level					
	Desirable		Borderline		High	
	mg/dl	mmol/l	mg/dl	m mol/l	mg/dl	m mol/l
Total cholesterol	<200	<5.17	200-239	5.17-6.19	>240	>6.20
LDL	<130	<3.36	130-159	3.36-4.12	>160	>4.13
HDL	>60	>1.55	35-59	0.90-1.54	<35	<0.90
Triglyceride	<200	<2.26	200-399	2.26-4.51	400-1000	4.53-11.29

Source: NCEP (2001)

Note: Total cholesterol = HDL + LDL + 20% TG

$$\text{LDL} = \text{TC} - \text{HDL} - \text{TG}/5 \quad (\text{for TG} < 400 \text{ mg/dl})$$

3. **Antioxidant**

3.1 Definition

An antioxidant is any substance that when present at low concentrations compared with those of an oxidizable substrate significantly delays or prevents oxidation of that substrate (Halliwell and Gutteridge, 1999). The term oxidizable substrate encompasses almost everything (except H₂O) found in foods and living tissues includes proteins, lipids, carbohydrates and DNA. The mechanisms of antioxidant activities show in Table 3. The most important mechanism is their reaction with lipid free radicals, forming inactive products. Additives with this mechanism are antioxidants in the proper sense. Usually, they react with peroxy or alkoxy free radicals, formed by decomposition of lipid hydroperoxides. Other inhibitors stabilize lipid hydroperoxides, preventing their decomposition into free radicals. Decomposition of hydroperoxides is catalyzed by heavy metals, and consequently metal chelating agents also inhibit oxidation. Some substances called synergists demonstrate no antioxidant activity in themselves, but they may increase the activity of true antioxidants. Another group of substances decompose lipid hydroperoxides by a non-radical pathway, thereby reducing free radical content. Finally, singlet oxygen oxidizes lipids many times faster than the common triplets oxygen, and consequently singlet oxygen quenchers also have an important inhibitory effect on lipid oxidation (Pokorny *et al.*, 2001).

Halliwell (2002) stated that dietary antioxidants maybe absorbed into the human body and might exert beneficial effects. This has been established most clearly for α -tocopherol and vitamin C. Specific uptake mechanisms for L-ascorbate exist in the human gastrointestinal tract. Absorption of all tocopherols in the diet occurs, but the liver selectively secretes α -tocopherol into the plasma (Traber, 1997). Evidence for the absorption of other plant phenolics is growing (Rice-Evan, 2000). For example, quercetin and catechins can be absorbed to some extent in humans; they and their metabolites can reach plasma concentrations in the range of 0.1-1 μ M

(Manach *et al.*, 1998; Donovan *et al.*, 1999). Such concentrations can delay the process of lipid peroxidation in liposomes, microsomes and low-density lipoproteins.

Table 3 Mechanisms of antioxidant activity

Antioxidant class	Mechanism of antioxidant activity	Example of antioxidant
Proper antioxidants	Inactivating lipid free radicals	Phenolic compounds
Hydroperoxide stabilizers	Preventing decomposition of hydroperoxides into free radicals	Phenolic compounds
Synergists	Promoting activity of proper antioxidants	Citric acid, ascorbic acid
Metal chelators	Binding heavy metals into inactive compounds	Phosphoric acid, Millard compounds, citric acid
Singlet oxygen quenchers	Transforming singlet oxygen into triple oxygen	Carotenes
Substances reducing Hydroperoxides	Reducing hydroperoxides in a non-radical way	Proteins, amino acids

Source: Pokorny *et al.* (2001)

3.2 Measuring Total Antioxidant Activity

3.2.1 The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) Method.

The 1, 1-diphenyl-2-picrylhydrazyl is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet colour, characterized by an absorption band in ethanol solution centered at about 520 nm (Molyneux, 2004). The DPPH method is one of the most studied methods to determine free radical scavenging activity. The parameter used in this method is EC_{50} which defined as the concentration of substrate that causes 50% loss of the DPPH activity (Brand-Williams *et al.*, 1995). The EC_{50} parameter also has the drawbacks. The higher the antioxidant activity, the lower is the value of EC_{50} . This is a widely used parameter to measure antioxidant power (Sahez-Moreno *et al.*, 1998).

3.2.2 Trolox-Equivalent Antioxidant Capacity (TEAC) Assay

The TEAC assay was first reported by Miller *et al.* (1993). TEAC assay is very popular method used to screen antioxidant. This assay is based on the scavenging of the relatively stable blue green ABTS radical (2,2'-azobis(3-ethylbenzothiazoline 6-sulfonate), converting it into a colorless product. The degree of this decolorization reflects the amount of ABTS radical that has been scavenged and can be determined spectrophotometrically (Arts *et al.*, 2003) at 414, 660, 734 and 820 nm. Rice-Evans and Miller (1994) explained that the radical was generated by the interaction of ABTS with the ferrylmyoglobin radical species, generated by the activation of metmyoglobin with H_2O_2 . The TEAC value is assigned by comparing the scavenging capacity of an antioxidant to that of trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid).

3.3 Effects of Antioxidant on Lipid Peroxidation

Lipid peroxidation is a model reaction of free radicals and active oxygen species. This reaction is important in, for example, food deterioration and oxidative modification of LDL which is now accepted as a key initial event in the progression of atherosclerosis. Lipid peroxidation proceeds by three different pathways: (1) non-enzymatic, free radical-mediated chain reaction, (2) non-enzymatic, non-radical oxidation and (3) enzymatic reaction. Lipid peroxidation can be inhibited by suppressing chain initiation and chain propagation and/or by enhancing chain termination. Metals often play an important role in radical generation. For this reason, proteins such as ferritin and ceruloplasmin, which sequester metal ions are also important antioxidants. Hydroperoxide and hydrogen peroxide are precursors of oxygen radicals and, hence, peroxidases such as glutathione peroxidase (GPX) also act as antioxidants. Superoxide dismutase (SOD) acts as an antioxidant by dismutating superoxide to triplet oxygen and hydrogen peroxide (Noguchi and Niki, 1999). Several factors in addition to efficiency in peroxy radical scavenging influence the ability of antioxidants to inhibit peroxidation in real lipids (membranes, lipoproteins, food lipids). Examples include partition coefficients (which govern the distribution of the antioxidant between the aqueous and lipid phases) and the ability to interact with any transition metal ions present. Thus, a direct test of antioxidant ability towards the lipid substrate of interest is often more informative than tests of the ability of an antioxidant to scavenge RO_2 radicals in isolation. A common test of the effectiveness of antioxidants is to measure their effects on the “peroxidizability” of LDL isolated from blood plasma after administration of the antioxidant to human subjects (Halliwell, 2002).

4. Saponins

1.1 Definition

Saponins are a vast group of glycosides, widely distributed in higher plants. Their surface-active properties are what distinguish these compounds from

other glycosides. They dissolve in water to form colloidal solutions that foam upon shaking (Tyler *et al.*, 1981). Saponins consist of a sugar moiety usually containing glucose (R), galactose, glucoronica scid, xylose, rhamnose or methylpentose, glycosidically liked to a hydrophobic aglycone called sapogenin (Francis *et al.*, 2002) as shown in Figure 6. Saponins can be classified into two groups based on the nature of their aglycone skeleton. The first group consists of the steroidal saponin, called neutral saponin (Figure 7(a)), which is almost exclusively present in the monocotyledonous angiosperms. The second group consists of the triterpenoid saponins, called acid saponins (Figure 7(b)), which are the most common and occur mainly in the dicotyledonous and giosperms (Bruneton, 1995). The aglycon may contain one or more unsaturated C-C bonds. The oligosaccharide chain is normally attached at the C₃ position (monodesmosidic), but many saponins have an additional sugar moiety at the C₂₆ or C₂₈ position (bidesmosidic). The great complexity of saponin structure arises from the variability of the aglycone structure, the nature of the side chains and the position of attachment of these moieties on the aglycone (Francis *et al.*, 2002). Experiments demonstrating the physiological, immunological and pharmacological properties of saponins have provoked considerable clinical interests in these substances.

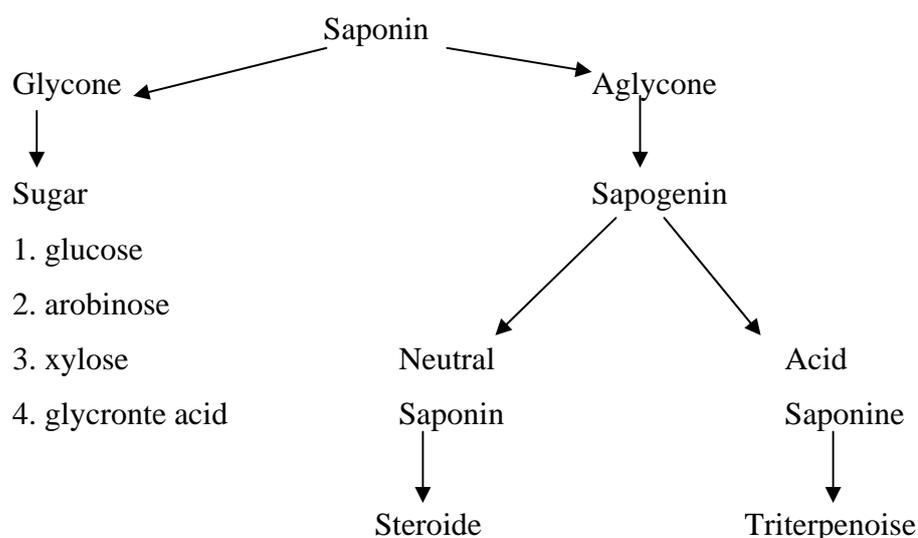


Figure 6 Classification of saponin.

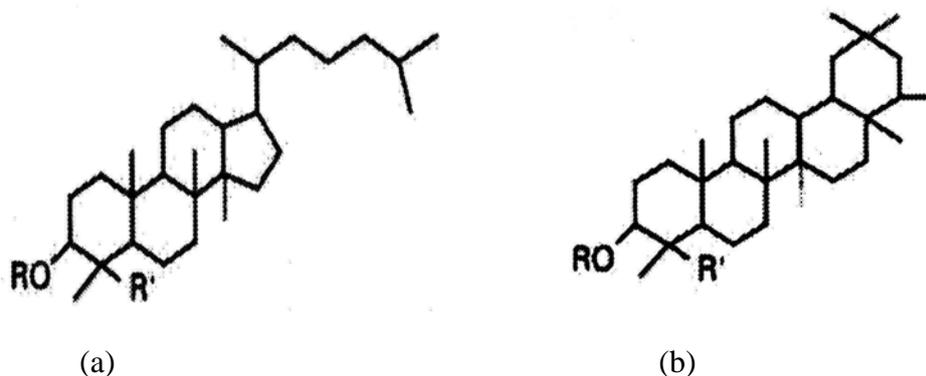


Figure 7 Basic structure of saponin aglycones: a steroid (a) and a triterpenoid (b).

Source: Francis *et al.* (2002)

4.2 Isolation and Characterization of Saponins

The modern methods available for the separation and analysis of saponin have been well reviewed by Marston *et al.* (2000), Muir *et al.* (2000) and Schopke (2000). There are several strategies available for the isolation of saponins. As a general rule, they begin with the extraction of the plant material with aqueous methanol or ethanol. Further processing of the extract is carried out after evaporation under reduced pressure, dissolution in a small amount of water and phase separation into n-butanol. It is currently recognized that this step is sometimes undesirable, since only those saponins with short oligosaccharide side chains will eventually be extracted into the butanolic phase. A further purification is then carried out, which involves liquid chromatography over a silica gel column, or a gradient elution from a polymeric support or liquid-liquid partition chromatography, or, as most commonly employed, High Performance Liquid Chromatography (HPLC) separation. In most cases, certain of the above steps have to be repeated with a change of support or eluent to achieve high purity. Once the saponin has been purified, it may be subjected to analytical methods including Mass Spectrophotometry (MS), proton and carbon NMR, and infrared spectroscopy. Other classical methods are used to ascertain the presence of saponins in a crude plant extract, and to elucidate their composition throughout purification steps. Thin Layer Chromatography (TLC) and

staining with dehydrating reagents containing aromatic aldehydes (such as anisyl aldehyde in sulfuric acid) are commonly used. The pure saponin may also be hydrolysed to verify the nature of its glycosidic moieties.

4.2 The Hypocholesterolaemic Effect of Saponins

Saponins form a heterogeneous group of triterpene or steroid glycosides which occur in many hundreds of plant species. Many of these are staple items of the human diet. Isolated saponins and foods containing saponins have been shown to lower plasma cholesterol concentrations in a number of animal species and it has been suggested that foods containing saponins could be important in formulation hypocholesterolaemic diets for human consumption. Thus the mechanism of the hypocholesterolaemic activity of saponins is of considerable interest. Sidhu and Oakenful (1986) had investigated the effects of different saponins on absorption of the bile salt sodium cholate from perfused loops of small intestine in the rat (in vivo). Purified saponins from soapwort (*Saponaria officinalis*), soya beans and quillaia (*Quillaia saponaria*) reduced the rate of absorption of the bile salt; soya-bean and soapwort saponins substantially but quillaia saponin was to a less extent. These results were explained by the formation of large mixed micelles by bile acid and saponin molecules in aqueous solution. These aggregates can have molecular weights in excess of 10^6 daltons, consequently the bile acid molecules incorporated in them are not available for absorption. Control of plasma cholesterol and nutrient absorption through dietary saponins could provide substantial health and nutritional benefits in humans. The effects of feeding alfalfa, on serum cholesterol and in modifying or preventing cholesterol induced atherosclerosis in rabbits, was studied by Cookson et al. (1967) and Cookson and Fedoroff (1968). Results showed that daily administration of large doses of cholesterol did not elevate serum cholesterol values above normal levels on alfalfa fed rabbits and there was no evidence of atherosclerosis. An effect of undigested alfalfa fiber preventing cholesterol absorption, was dismissed because there was no relationship between dose of cholesterol and dose of alfalfa. There were some components in alfalfa which blocked the absorption mechanism for cholesterol in the intestinal mucosa. Barichello

and Fedoroff (1971) found that cholesterol fed rabbits with ileal bypass required less alfalfa to prevent serum cholesterol elevation, than rabbits with normal gut length. These related to the reduced intestinal absorptive surface area, supporting the hypothesis of some form of complex formation. Extensive experimentation by Malinow *et al.* (1977, 1979, 1980), with respect to alfalfa, confirmed that alfalfa saponins, not just alfalfa fibre, was responsible for reducing cholesterol absorption. In another study, Parab and Mengi (2002) conducted saponin extract from *Acorus calamus L.* (Araceae) testing for hyperlipidemic activity. Results showed that saponins (10 mg/kg) could decrease the serum cholesterol and triglyceride levels significantly. However, neither of the lipoprotein levels were brought down to baseline values, but it was still concluded that the saponin contribute to the hypolipidemic activity of *A. calamus*. Saponin fraction from *Allium* species have been shown to decrease the plasma total cholesterol levels (Matsuura, 2001). The active fraction contained steroid saponins which were thought to be responsible for the cholesterol lowering effects of garlic. Overall, the works done in relation to the hypocholesterolaemic effect of saponins confirm antihypercholesterolaemic activities in animals that are shown in Table 4. Milgate and Roberts (1995) had summarized the mechanisms by which saponins may reduce cholesterol levels were:

1. The formation of insoluble addition complexes with β -hydroxysteroids, decreasing intestinal absorption of cholesterol, producing an increase in faecal sterol excretion.
2. Adsorption of bile acids to dietary fibre was enhanced in the presence of saponins, forming large molecular weight micelles, which excluded the bile acids from reabsorption. The ensuing loss of bile acids, was offset by the enhanced conversion of cholesterol to bile acid in the liver, resulting in hypocholesterolemia.
3. Interaction with intestinal mucosal cells leading to an increase in cell permeability and subsequent rapid loss of normal cellular function, increasing exfoliation and promoting proliferation. The increased loss of gut cells contributed to an additional increase in faecal cholesterol excretion.

4. Their content of β -1-4 linked sugars increasing soluble fibre intake and promoting volatile fatty acid (VFA) production in the large bowel resulting in decreased hepatic cholesterol output.

Table 4 Review of saponin research with rats and in vitro

Study	Saponin type	Treatment(diet)	Animal/ treatment	Duration time	Effects/Findings
Coulson and Evans (1960)	Quillaja Saponin white (Gypsophila)	Basal + various supplements Saponin 2 g/100g diet	Rat exp. x 12	4-6 week	Both saponins depressed weight gain. Quillaja saponin reduction was reversed by adding of cholesterol. The essential fatty acid, linoleic, when esterified or protected by antioxidant, increased rate of gain, whereas free linoleic acid had no effect.
Topping <i>et.</i> <i>al.</i> (1978)	Purified saponin	Control diet (CD) CD +1% saponin CD +1% cholesterol CD+saponin+cholesterol	Rat 4x5	3 week	Saponin diet caused an increase in faecal bile acids and neutral sterols, supporting hypothesis that saponins bind bile acids and prevent reabsorption.
Oakenfull <i>et.al.</i> (1979)	Saponin white	Control Diet+1% saponin Diet+1% cholesterol Diet+1% cholesterol +1% saponin	Rat 4x5	3 week	Dietary saponins increased the excretion of bile acids and neutral sterols in the faeces and they induced a further increase in the rate of bile acid secretion when this was stimulated by a high cholesterol diet.

Table 4 (continued)

Study	Saponin type	Treatment(diet)	Animal/treatment	Duration time	Effects/Findings
Topping <i>et.al.</i> (1980)	Quillaja Soya saponin	Pure saponin extract 1% of diet	Rat	3 week	Soya saponins resulted in increased bile acids and neutral sterol excretion in rats on a high cholesterol diet. Quillaja saponins primarily increased neutral sterol excretion. The higher cholesterol excretion was due to quillaja saponins forming complex with dietary cholesterol. Soya and saponin with did not.
Malinow <i>et.al.</i> (1981)	Alfalfa	Semipurified diet Diet+1% alfalfa top saponin Diet+2% alfalfa top saponin All diet+1% cholesterol	Rat 3x8	6 month 2 month	Long term feeding of alfalfa top saponins had no effect on growth/survival. Serum cholesterol & triglycerides decreased 1% in 6 month group. Saponins showed no evidence of toxicity.
Calvert and Yeates. (1981)	Soya Alfalfa	Soya bean flour Wheatbran Lucerne <i>Pinus insignis</i> sawdust	In vitro		Removal of saponin from soy flour did not affect bile salt adsorption. Bile salts were adsorbed onto lignin & wheatbran (deficient in saponin).

Table 4 (continued)

Study	Saponin type	Treatment(diet)	Animal/ treatment	Duration time	Effects/Findings
Oakenfull and Sidhu. (1983)	Saponin white	Saponin white	In vitro		Formed large mixed micelles with bile salts in vitro, reducing the rate of dialysis of sodium cholate by 73%.
Yamamoto <i>et.al.</i> (1983)	Ginseng (ginsenoside) Fraction 3 Fraction 4	Chow Chow+1mg/100g body wt. Of spn, orally after tumour transplant	Rat 8x12	5 to 12 day	Plasma cholesterol, triglycerides & non-esterified fatty acid levels were reduced with oral administration. Incorporation of ¹⁴ C-acetate into total lipid, free & esterified cholesterol, triglycerides&phospholipids in liver was enhanced by fractio 3, in both normal & tumour bearing rats.
Yamamoto <i>et.al.</i> (1983)	Ginseng (ginsenoside saponin) fraction 4	Chow Chow + 10 mg/100g body wt.of saponin intramuscular injected	Rat 2x8	Daily/ 2 wk	Evlevation of plasma cholesterol and triglycerides was reduced. Elimination of ¹⁴ C-cholesterol from plasma was accelerated as was faecal excretion of ¹⁴ C bile acids and ¹⁴ C sterols, by administration of fraction 4 saponins.

Table 4 (continued)

Study	Saponin type	Treatment(diet)	Animal/ treatment	Duration time	Effects/Findings
Story. <i>et.al.</i> (1984)	Alfalfa	Alfalfa plant 147g/100g Alfalfa saponin 147g/100g	Rat x 30	4 week	Alfalfa plant saponin bound sig. Quantities of cholesterol. Alfalfa sprout saponins interacted with cholesterol & inhibited growth <i>Trichodermivirde</i> (measure saponin/cholesterol interaction). Liver cholesterol accumulation in cholesterol fed rats was not reduced. Serum cholesterol reduced alfalfa sprouts greater than alfalfa plant material. Increased faecal sterol excretion.
Johnson. <i>et.al.</i> (1986)	Gypsophila Saponin Saponaria Soya saponin Tomatine	Gypsophila Saponin Saponaria Soya saponin Tomatine	In vitro (Rat intestine)		Gypsophila inhibited carrier mediated galactose transport, but 1-galactose passive transport increased. Saponaria& tomatine decreased transmural potential diff.(PD). Soy saponin less effective, reduced permeability barrier to sodium at the brush border, thus discharging the electrochemical radient& removed the driving force for sugar transport.

Table 4 (continued)

Study	Saponin type	Treatment(diet)	Animal/ treatment	Duration time	Effects/Findings
Sharma. (1986)	Feungreek seed	8 x diet 4.8% saponin	Rat 8x10 1x20	8 week	Saponins lowered serum cholesterol as well as preventing elevation when fed with hyper cholesteremic inducing diet. Crude saponin showed hypocholesterol activity. Truglyceride levels were not affected.
Gee and Johnson (1988)	Gypsophila saponin	Diet Diet + 1.5% saponin	Rat 2x10	7 day	Serum cholesterol Reduced in saponin fed rats & cholesterol Content of fecal contents increased. Loss of cholesterol via exfoliated mucosal cells may contribute to hypocholesterolaemic effect. Changes in villus morphology were observed. No evidence of inflammatory of functional damage to jejunal mucosa.

Table 4 (continued)

Study	Saponin type	Treatment(diet)	Animal/ treatment	Duration time	Effects/Findings
Southen <i>et.al.</i> (1988)	Gypsophila saponin	Basal Basal + 0.02% saponin Low iron diet Low iron diet+saponin Low zinc diet Low zine diet+saponin	Rat 6x10	2 day	Zn status/femur concentration not adversely affected. Sig. reduction in blood cholesterol in both low iron groups. Significant reduction in iron status in basal+ saponin & low iron+ saponin groups, suggest saponin-mineral interaction complex.
Amigo. <i>et.al.</i> (1992)	Bean saponin fraction	Control Fraction I Fraction II Fraction III Diosgenin	Rat 5x5	5 to 6 day	The effect of bean on serum, biliary cholesterol & phospholipids outputs & on hepatic cholesterol ester concentration are dependent on the starch fraction of beans, which contain saponin content.

Table 4 (continued)

Study	Saponin type	Treatment(diet)	Animal/ treatment	Duration time	Effects/Findings
Stark and Madar. (1993)	Fenugreek ethanol extract	Diet Diet + cholesterol Diet +cholesterol +Ethanol extract	Rat 3x10	4 week	Addition of ethanol extract from fenugreek seeds affects cholesterol metabolism in rats. Lower liver and plasma cholesterol concentrations may have been caused by increased excretion of fecal bile acids and a subsequent increased conversion of cholesterol to bile acids.
Jenkins and Atwal. (1994)	Saponin	Dietary saponin Vitamin A & E	Chick		Saponin-induced reduction serum cholesterol occurred only when a hypercholesterolaemic diet had been fed. There is also evidence of increased cholesterol synthesis to compensate for saponin-induced excretion.

Table 4 (continued)

Study	Saponin type	Treatment(diet)	Animal/ treatment	Duration time	Effects/Findings
Pillion <i>et.al.</i> (1996)	Quillaja saponin	Saponin extract	In vitro		The efficacy as absorption-enhancing agents across nasal mucosa in rats was greatest in those Quillaja saponins with the lowest surfactant strength and haemolytic titres.
Morehouse <i>et.al.</i> (1999)	Natural saponin Synthetic saponin	Natural saponin Synthetic saponin	In vitro		The mechanism of action of saponin was luminal but did not involve stoichiometric complex ratio with cholesterol. The synthetic saponins tiqueside and pamaqueside were much more potent than naturally occurring saponin such as those from Lucerne in preventing hypocholesterolaemia and that the in vivo potency of pamaquecide was 10-fold that of tiqueside even though it differs from tiqueside only by an additional keto group.

Table 4 (continued)

Study	Saponin type	Treatment(diet)	Animal/ treatment	Duration time	Effects/Findings
Choi <i>et.al.</i> (2001)	Ginsenosides (from <i>Panax quinquefolius</i> , <i>P. Japonicus</i>) <i>Styrax paponica</i>	Ginsenosides	In vitro		Cholesterol enrichment was shown to have an inhibitory effect on many membrane ATPases, as it may directly interact with the boundary lipids of ATPase and alter the intermolecular hydrogen bonds of the protein. Ginsenosides share the steroid backbone and amphipathic nature with cholesterol. The desacyl-jego-saponin (from <i>S. japonica</i>) and gensenoside-Rd, which had little or no effect on membrane permeability, were capable of stimulating Na ⁺ - Ca ⁺ exchange activity in canine cardiac sarcolemmal vesicles.

5. Technique in Product Development System

5.1 Definition of Product Development

Product development system is very important in the food industry, as in all other industries. The concept of product development is the co-ordination of the different research techniques- marketing, processing and engineering- into one type of research which aims to develop new products (Earle, 1985). The two parts of product development- the knowledge of the consumer's needs/wants and the knowledge of modern scientific discoveries and technological developments- are both equally important. The product development process coordinates the specific research activities such as product design, process development, engineering plant design, marketing strategy and design with the aim of producing an integrated approach to the development of new products. Thus, the product development process combines and applies the natural sciences with the social sciences to systematically produce innovation in industry (Earle *et al.*, 2001).

5.2 Product Development Process

Earle and Earle (1999) suggested a simple version of four stages of product development process as following:

Stage 1: Product strategy development. The product strategy starts with the finalizing of the product development strategy and product development programme. The aims of the individual product development projects can be set. The project starts with the generation of product ideas and the outlining of the product design strategy and ends with the product concept and product design specifications. The substates in stage 1: product strategy for the individual project are: defining the project; developing the product concept; identification of processes, distribution and marketing; development of product design specifications; planning of the project; predictions of project cost and financial outcomes.

Stage 2: Product design and process development. The themes for stage 2 are integration, creativity, systematic planning and monitoring. Food product development is process-intensive, the characteristics of the product are highly constrained by the processing, Therefore the process and the product are developed together. The product prototype is developed by many techniques such as sensory methods, product optimization or response surface methodology (Rudolph, 2000). Five important outcomes of this stage are clearly defined final product prototype with consumer acceptance; product specifications including processing method, physical distribution; market strategy including distribution, promotion, pricing; prediction of investment needed and financial outcomes; probability of achieving project completion and financial outcomes.

Stage 3: Product commercialization. Product commercialization is full scale-up of both production and marketing. These two developments need to be integrated throughout product commercialization. There are four important stages in product commercialization: setting up the commercialization; design of marketing, production and distribution; testing of marketing, production and distribution, final integration of marketing, production and finance.

Stage 4: Product launch and evaluation. Effective product launch is a key driver of top performance. Despite its importance, costs and risks, product launch has been relatively under-researched in the product literature (Di Benedetto, 1999). There are three important parts of the launch-strategy, activities and demand outcomes (Guiltinan, 1999) Finally, the evaluating and controlling the product launch is critical to success.

At the end of the product development stage, the alternate product prototypes have usually been narrowed down to a manageable number of final prototypes. Consumer acceptance tests which participants may be composed of 100-500 consumers, give and estimate of product acceptance in different areas around the country (Resurreccion, 1998).

5.3 Consumer Study in Product Development

Consumers are the centre of product development in the food industry and consumer are the final arbiters on food product acceptance. It is important in product development to understand basic consumer behavior and food choice as well as the individual product/ consumer relationship (Earle, 1997). Consumer testing is necessary throughout the various stages in the product cycle (ASTM, 1979). These stages include the development of the product itself, product maintenance, product improvement and optimization and assessment of market potential.

Consumer studies can be classified into two categories: qualitative and quantitative consumer research. Qualitative consumer research methods are useful in defining critical attributes of a product and focus group discussion is the most commonly used for qualitative method. Quantitative research involves measurements and the methods are preference test and acceptance test. Resurreccion (1998) described consumer test procedure into two parts. One is project planning and the other is product evaluation and data collection. Project planning includes the definition of objectives, selection of an appropriate test and experimental design, identification and recruitment of the consumer sample, scheduling and implementation of the test, data processing and analysis methods, interpretation and reporting of results in a timely manner. Product evaluation and data collection include maintaining appropriate test controls, writing instruction and briefing, start and completion dates, duplication of forms, rewards and incentives, data collection, analysis and processing.

5.3.1 Focus group discussion

The focus group is a method by which small groups of consumers (8-12 participants) are used to obtain information about their reaction to products and concepts and to investigate various other aspects of respondents perceptions and reactions. This method is used to determine product attributes that consumers think are important and should be maximized in the product and characteristics that

consumers do not like and think should be minimized or eliminated from the product (Resurreccion, 1998). The moderator presents the subject of interest and facilitates the discussion using group dynamics techniques to uncover as much specific information from as many participants as possible directed toward the focus of the session (Meilggard *et al.*, 1999). Although focus group are loosely structured, the format take the structure described: In a focus group, 8-12 people sit around a table, with a moderator who leads the discussion. The discussion lasts from 90-120 minutes. Focus group procedures are the introduction (10 min), rapport/reconnaissance (20 min), in-depth investigation (60 min) and closure (10 min) (Galvez and Resurreccion, 1992).

5.3.2 Consumer Survey

Consumer survey is a survey research. Survey is a technique for gathering information from a large number of users (Brehob, 2001). The survey design consisted of 7 steps; establish the goals of the project, determine the sample, choose interviewing methodology, create the questionnaire, pre-test the questionnaire, conduct interviews and enter data and analyze the data (Trochim, 2000; Creative Research Systems, 2005). The key step in designing a survey was setting the goals. The goals of the survey determined the target population and questions. If the goals were not clear, the result of the survey would be uncertain. Correctly determining the target population was critical; it should represent the targeted users of the interface and bias should be eliminated. This concept was known as sampling. Sampling is defined as “the act, process, or technique of selecting a representative part of a population for the purpose of determining parameters or characteristics of the whole population (Kuter and Yilmaz, 2001).

The equation used for sample size calculation was (Aaker and George, 1983; Narins, 2002).

$$n = P(1 - P) / \text{Standard error}^2$$

where n = sample size and P = the population proportion. For a confidence interval of 95 percent, the standard error multiplied by 1.96 is the sampling error (Narins, 2002).

Questionnaire defines as a form that people fill out, used to obtain demographic information and views and interests of those questioned (Brehob, 2001). Kirakowshi (1998) defines a questionnaire in a more structural way as a method for the elicitation, and recording and collecting information. Questionnaire designed with three type of questions; namely multiple choices, numeric open-end and text open-end. Mainly, there are two types of response format: structured response and unstructured response (Trochim, 2000). Structured responses were very easy to be answered by the respondents but might not capture everything in the respondents' mind(s) (e.g. responses to multiple-choice questions). In unstructured responses, the respondents write down text as a response (e.g. responses to text open end questions). Questions should be clear and unambiguous. Also, the order of the questions matters. For example, the easier questions should be placed before the harder questions. The rationale behind this is to prevent respondent boredom at the beginning and to motivate them to complete the survey (Kuter and Yilmaz, 2001).

5.3.3 Acceptance test

Acceptance tests measure acceptability or liking for a food by consumers. The methods most frequently used to determine quantify acceptance is the 9-point hedonic rating. The 9-point hedonic scale has been used for a number of years and is validated in the scientific literature (Stone and Sidel, 1993). Typically a hedonic test today would involve a sample of 75 to 150 consumers who are regular users of the product. Samples are served to panelists monadically (one at a time) and the panelists are asked to indicate their hedonic responses to the sample on the scale (Lawless and Haymann, 1998).

5.3.4 Data Analysis: Multivariate Analysis

Multivariate method is the technique that analyzed the relationship between independent variables and dependent variables more than two variables in the same time. In a multivariate data set, the response variables could be continuous, binary, discrete, categorical or any mixture of these, while the individuals or units may be experimental plots, objects, patients, animals, skulls, and so on (Haslett, 2001). Multivariate analysis is better suited for studying the data relationship between consumer evaluations and descriptive analysis results or physicochemical measurement (Resurreccion, 1988). There are many methods in multivariate techniques such as factor analysis and discriminant analysis.

5.3.4.1 Factor Analysis

Factor analysis (FA) is a technique that is most commonly used in food quality studies for data reduction and simplification. The method is used to reduce a large number of variables to a smaller set of new variables, called factors, which can be used to explain the variation in the data. The objective in factor analysis is to find a smaller number of factors that together can replace the original variables measured in the study. In factor analysis, the factors are obtained by algorithms that work with correlations of variables as opposed the variances, which are commonly used in PCA (Principal Components Analysis). In many cases, the axes found by factor analyses are treated by the mathematical operation called “rotation”. The rotated axis yields a better alignment with the original axes. It is therefore possible to make a clearer interpretation of the resulting pattern of data points (Resurreccion, 1998).

Factor analysis and Principal component analysis are similar in many ways, the major similarities being that both methods make use of the correlation (variance-covariance) among attributes, and both methods have the objectives of reducing the number of attributes into a new set of attributes, the so-called factors or components (Gacula, 1997). There are two important differences

between PCA and FA. 1) PCA produces an orthogonal transformation of the original variables with no underlying statistical model; the new variables (principal components) are obtained to explain the variances of the original variables. FA is based on a proper statistical model and is more concerned with the covariances between the original variables than with explaining the variances. 2) Whereas PCA tries to explain all the variation in the data, FA tries to explain only the common or shared variation. This means that FA is likely to be more useful than PCA when measurement accuracy is low (Haslett, 2001).

5.3.4.2 Discriminant Analysis.

Discriminant analysis is a multivariate technique aimed at determining which set of variables best discriminates one group of objects from another (Resurreccion, 1988). The researcher is interested in understanding group differences or in predicting correct classification in a group based on the information on a set of variables or when probabilities of group membership must be determined. The discriminant functions can be used to predict the acceptability of a given sample through the calculation of posterior probabilities of membership to either the acceptable or unacceptable group (Frank *et al.*, 1990). In food quality measurements, the predictor variables are both instrumental measurements or sensory attribute ratings from descriptive analysis tests, or both, and the food products are grouped into acceptable (hedonic score of six and above) or not acceptable (below six), as determined through a consumer acceptance test.

5.3.4.3 Logistic Regression

Binomial (or binary) logistic regression is a form of regression which is used when the dependent is a dichotomy (0 and 1) and the independents are of any type. Multinomial logistic regression exists to handle the case of dependents with more classes than two. When multiple classes of the dependent variable can be ranked, then ordinal logistic regression is preferred to multinomial logistic regression. Continuous variables are not used as dependents in logistic

regression. Unlike logit regression, there can be only one dependent variable. Logistic regression can be used to predict a dependent variable on the basis of continuous and/or categorical independents and to determine the percent of variance in the dependent variable explained by the independents; to rank the relative importance of independents; to assess interaction effects; and to understand the impact of covariate control variables (Garson, 2006). It is defined as a logistic transformation of p , called logit of p . Logit (p) is the log (to base e) of the odds or likelihood ratio that the dependent variable is 1.

$$\text{logit}(p) = \log(p / (1-p))$$

It follows that logistic regression involves fitting to the data and equation of the form:

$$\text{logit}(p) = a + b_1X_1 + b_2 X_2 + b_3 X_3 + \dots$$

The meaning of the coefficients b_1, b_2 (Lea, 2006).

The most common way of interpreting a logit is to convert it to an odds ratio using the $\exp(\beta)$ function. One can convert back using the $\ln(\)$ function. Note that an odds ratio above 1.0 refers to the odds that the dependent = 1 in binary logistic regression. The closer the odds ratio is to 1.0, the more the independent variable's categories (ex., male and female for gender). There are independent of the dependent variable, with 1.0 representing full statistical independence (Garson, 2006).

$$\text{Odds ratio} = p / 1-p$$

5.3.4.4 McNemar Chi-squareTest

McNemar test is a within-subjects version of the chi-square test. Imagine a study in which participants repeated the same measure twice,

and the measure is a dichotomous one using yes/no answers. McNemar's chi-square can use a simple computational formula.

		<u>Time 2</u>	
		<u>No</u>	<u>Yes</u>
<u>Time 1</u>	<u>No</u>	a	b
	<u>Yes</u>	c	d

$$\text{McNemar's } \chi^2 = \frac{(c - b)^2}{c + b}$$

This is a d.f.=1 test, and the critical value at $\alpha = 0.05$ is 3.84 (Newson, 2006).

5.4 Sensory Descriptive Analysis

Descriptive analysis methods involve the detection (discrimination) and the description of both the qualitative and quantitative sensory aspects of product by trained panels of 5 to 100 judges (Meilgaard *et al.*, 1999). Stone and Sidel (1985) defined descriptive analysis as the process of describing the perceived sensory characteristics of a product, usually in the order of their occurrence during evaluation. The applications of sensory descriptive analysis are defining the sensory properties of a target product for new product development (Szczesniak *et al.*, 1975), defining the characteristics/ specifications for a control or standard for QA/QC and R&D application, document product attributes before a consumer test, tracking a product's sensory changes over time and mapping perceived product attributes for the purpose of relating them to instrument, chemical or physical properties (Moskowitz, 1979). The sensory descriptive analysis consists of the qualitative aspect in characteristics, the quantitative aspect in intensity, the time aspect in order of appearance and the integrated aspect in overall impression (Meilgaard *et al.*, 1999).

Gacula (1997) presented descriptive sensory analysis methods; flavor profile and profile attribute analysis, Quantitative Descriptive Analysis, Spectrum

Descriptive Analysis Method and Variants of Descriptive Analysis (Free-Choice Profiling).

The Quantitative Descriptive Analysis (QDA) method was developed by Tragon Corp.(Stone *et al.*, 1974). This method relies heavily on statistical analysis to determine the appropriate terms, procedures and panelists to be used for analysis of a specific product. Panelists are selected from a large pool of candidates according to their ability to discriminate differences in sensory properties. The training of QDA panels requires the use of product and ingredient references to stimulate the generation of terminology. Attention is given to development of consistent terminology, but panelists are free to develop their own approach to scoring, using the 15 cm (6 in.) line scale. QDA panelists evaluate products one at a time in separate booths to reduce distraction and panelist interaction. The results of a QDA test are analyzed statistically and the report generally contains a graphic representation in form of a “spider web” (Meilgaard *et al.*, 1998). The applications of QDA were benefit to research and development, production and quality control, market research and consumer study (Stone *et al.*, 1997).

5.5 Product Optimization

Optimization can be defined as “a procedure for developing the best possible product in its class or category” (Stone and Sidel, 1983). Optimization is a series of steps for obtaining the best result under a given set of circumstances (Cacula, 1993). Most food products contain numerous ingredients and their manufacture typically involves several different processing steps. All ingredients and processing steps are not equally important to acceptability of the product. Therefore, optimization is used to identify those variables or combinations of variables that are important to sensory acceptance, then to determine a degree and level of importance of each to acceptance and then to predict that combination of independent variables that will yield optimum acceptance.

There are five basic steps in optimizing a product formulation (Fishken, 1983). First is the ingredient screening, a formulated product consists of ingredients for which there are numerous options and suppliers. The final set of ingredients for the finished product should be selected through consumer research. The second is identification of high-impact ingredients. There are ingredients that, when varied, have a strong impact on the overall sensory properties, consumer acceptance or cost of the product. The third step is the design of test products. The most critical step is to set the ingredient levels for the design of test products. If there is a true optimal formulation, then there must be ingredient levels that are higher or lower than the optimal value. For example, the central-composite design calls for 3-5 levels of each ingredient for the design of test products. The fourth step is consumer testing. There is no magical number of respondents that is correct for consumer tests, but a minimum of 100 subjects is recommended (Resurreccion, 1988). The final step is data analysis. Several approaches (e.g., multiple regression analysis, incomplete fractional factorial design in conjunction with response surface methodology (RSM), mixture design) can be used to identify properties and levels which are most likely to be important to acceptance.

Moskowitz (1994) suggested a standard sequence set of steps to optimize a product. Step 1 is selection of relevant variables and layout of levels by experimental design. Step 2 is questionnaire development for testing among respondents. Step 3 is test implementation. Step 4 is data analysis and database development. Step 5 is creation of models from the empirical data. And step 6 is use of the model for predictions of product fitting specific goals.

6. Drying Process

6.1 Definition

Drying or dehydration is defined as that unit operation which converts a liquid, solid or semi-solid feed material into a solid product of significantly lower moisture content. Drying preserves food by lowering the amount of water or moisture

in the food material. This is necessary to prevent microbial growth and chemical reactions which can only occur when enough water presents in the food. Equilibrium Relative Humidity (ERH) is a term used to describe the availability of the water to the chemical environment and microorganisms present in foods. The equilibrium relative humidity or percent ERH scale varies from zero to 100 (Wolf *et al.*, 1990). In most cases, drying involves the application of thermal energy, which causes water to evaporate of thermal energy, which case water to evaporate into the vapour phase. Drying is a complex process involving simultaneous coupled, transient heat, mass and momentum transport. These are often accompanied by chemical or biochemical reactions and phase transformations, such as glass transition and crystallization, along with the shrinkage (Mujumdar, 1997).

Food and dried commercially, starting either from their natural state such as vegetables, fruits, grains, herb and spice. In addition to preserving the product and extending its shelf life, drying may carried out the objectives: to obtain desired physical form (e.g. powder, flakes, granules), to obtain desired color, flavor or texture and to reduce volume or weight for transportation. The quality changes in food during drying are divided into three aspects; the physical changes e.g. shrinkage, loss of density, crystallization, change of solubility, alteration of shape, size, porosity, loss of aroma, organoleptic properties, the chemical changes e.g. loss of chemical activity, browning reation, lipid oxidation, discoloration and nutritional changes e.g. loss of vitamins and proteins (Bluestein and Labuza, 1975; Adamice *et al.*, 1995).

6.2 Kinetics of Drying

The mechanism of drying was related to psychrometry, equilibrium moisture content, water activity and sorption isotherm. Fellows (1996) described that initially the moisture decreased rapidly because the water moved from the interior at the same rate as it evaporated from the surface which was in constant-rate period. The factors were dry-bulb temperature, relative humidity, and air velocity. The drying rate remains constant until critical moisture content, after that it declines until the equilibrium which called the falling-rate period. Mujumdar (1997) described the

drying kinetic which consider the drying of a wet solid under fixed drying conditions. After an initial period of adjustment, the dry-basis moisture content, X , decreases linearly with time, t , following the start of the evaporation. It is followed by a non-linear decrease in X with t until, after a very long time; the solid reaches its equilibrium moisture content, X_e and drying stops. In terms of free moisture content, defined as

$$X_f = (X - X_e)$$

the drying rate drop to zero at $X_f = 0$.

By convention, the drying rate, N , is defined as:

$$N = \frac{-M_s dX}{A dt} \quad \text{or} \quad \frac{-M_s dX_f}{A dt}$$

under constant drying conditions. Here N ($\text{kg m}^{-2} \text{ h}^{-1}$) is the rate of evaporation of water, A is the evaporation area (this may be different from the heat transfer area) and M_s is the mass of bone dry solid. If A is not known, then the drying rate may be expressed in kg water evaporated per h.

The mechanism underlying this phenomenon depends both on the material and the drying condition. Many foods and agricultural products do not display a constant rate period at all since internal heat and mass transfer rates determine the rate at which water becomes available at the exposed evaporating surface (Mujandar, 1997).

6.3 Hot Air Drying

There are four phases of hot air drying. First phase is the raising of core temperature. In the first phase of raising the core temperature, the product is warmed as fast as possible, without case hardening the product, to within 10 to 20 degrees of the process air temperature. In the counter flow configuration, the wet fruit is placed

in the cool end and is subjected to very wet air that has lost 20 degrees or more by passing through the length of the Tunnel. This wet air transfers heat very fast and as the car moves forward in the dryer, the process air temperature rises and humidity drops. This accelerates the transition to the second phase. In the Parallel flow configuration, the wet car is placed in the hot end and the product is immediately subjected to the high temperatures and low humidity of the high-pressure end. Rather than pulling the product when it is dry (counter flow), parallel flow requires that in less than two hours another car must be placed in the hot end to prevent the previous car from case hardening. Thus the wet product drives the dehydration rather than the dry product. As each car is placed in the high-pressure end, a charge of wet cool air bathes all of the cars behind it for a few minutes. This dehydration and re-hydration cycle continues throughout the process.

Second Phase is: rapid dehydration. In the second phase the moisture content of the product is in near free fall. To maximize production, moisture inside the dryer needs to be controlled. As a rule the moisture content of the process air when drying most products, measured at the high-pressure end, should be 17% to 19%. After the air passes through the dryer the relative humidity at the cool end should be 35% to 50%. Remember, each product is different and should be treated as such.

Third Phase is transition. Transition is the most critical phase, in regards to possible damage to the product. The high rate of moisture release experienced in the second phase slows down to a crawl. Most of the water in the product is gone. Capillary action at the cellular level now provides the majority of the free water being driven off. The evaporative cooling that has kept the core temperature of the product well below the process air temperature slows as well. Case hardening, cooking, and caramelization are all very possible as the product passes through the transition phase.

Fourth Phase is baked out. The final phase is characterized by a slow reduction in the product moisture content. This phase is normally the longest, and depending upon the target moisture content, may include over 1/2 the dwell time.

Carmelization is still a threat in the last phase, as well (Commercial Dehydrator Systems Inc., 2000).

6.4 Microwave Drying

Microwave is forms of electromagnetic energy and it transmitted as waves, which penetrate food, and then converted to heat (Fellows, 1996). Vacuum microwave drying offers an alternative way to improve the quality of dehydrated product (Lin *et al*, 1998). A prerequisite for the absorption of microwave energy by the material is the existence of substances containing dipoles or dipolic regions (polar centers). The heat produced in the product is directly transferred by means of conduction mechanisms throughout the entire mass of the radiated material (Drouzas and Schubert, 1996). The conversion of electromagnetic energy into thermal energy is realized because of the electromagnetic characteristics of materials. It depends principally on the material, temperature and frequency. And three parameters- conductivity, permeability and permittivity are required to describe any material (Linn High Therm Gmbh, 2000). Since the electromagnetic energy is dissipated directly in the dried material, heat losses are considerably reduced. The drying rate of microwave drying was significantly higher than convection drying (Figure 8) and the moisture content could reduce faster, so it could save the process time (Figure 9) (Kudra and Mujumdar, 2002). Thus, the performance of microwave drying is better than that of convective drying.

The drying curve was using a simple diffusion model (Equation 1) by Maskan (2000).

$$MR = \frac{X - X_e}{X_0 - X_e} \quad (\text{Equation 1})$$

Which

MR = moisture ratio

- X = moisture content (g water / g dry solid) at any time
 X_e = equilibrium moisture content (g water / g dry solid)
 X_0 = initial moisture content (g water / g dry solid)

For microwave drying it can be assumed that $X_e = 0$ (Maskan, 2000).

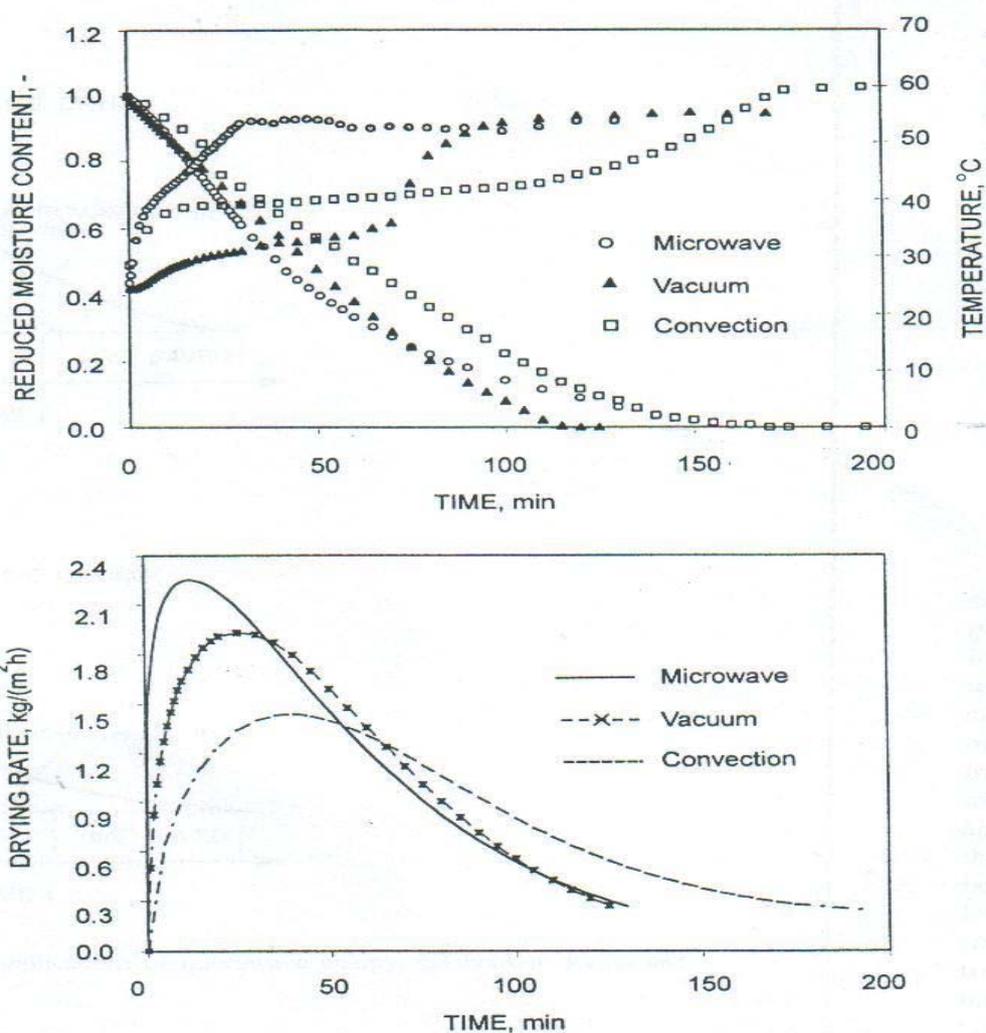
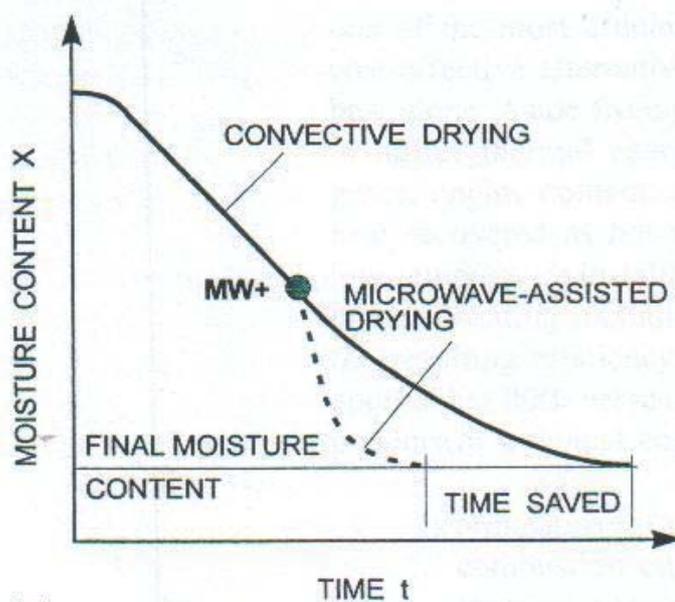


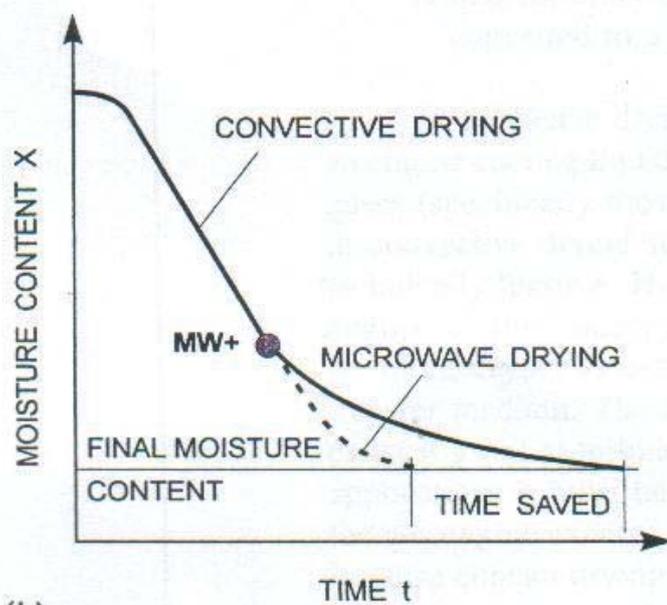
Figure 8 Convective, vacuum and microwave drying of chlorpropamide.

$$T = 60^{\circ}\text{C}, P = 0.1 \text{ atm}, u = 5.4 \text{ m/s}, P' = 385 \text{ W/kg.}$$

Source: Kudra and Mujumdar (2002).



(a)



(b)

Figure 9 Typical applications of microwave energy.

(a) booster drying and (b) finish drying

Source: Kudra and Mujumdar (2002)

Microwave-vacuum drying offers an attractive, cost-effective alternative to freeze-drying in the production of high-quality heat-sensitive products. Microwave drying under vacuum appears to be the solution of choice as it offers all advantages of dielectric heating but at a reduced processing temperature being the function of operating pressure (Figure 10). In addition, a volumetric heat transfer mechanism coupled with drying in the absence of oxygen (vacuum) provides an ideal low-temperature drying technique (Kudra and Mujumdar, 2002). The microwave vacuum dryers are in commercial production, manufacturing fruit juice concentrates, tea powder and enzymes. Pilot-plant tests have also been successfully performed for drying such vegetables as mushroom, onions and asparagus (Mujumdar, 1995).

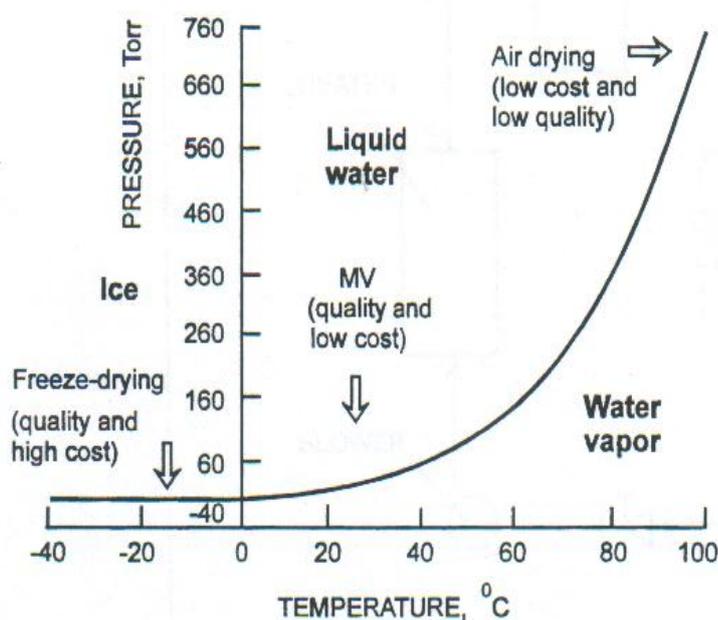


Figure 10 Microwave-vacuum drying versus air and freeze-drying

Source: Kudra and Mujumdar (2002)

MATERIALS AND METHODS

Materials

1. Material

- Raw Material. Jiaogulan from the Royal Pangda Station in Amphur Samern, Chiangmai, Thailand, was used for this research. The plants were harvested in the early morning before being transported to the Royal plant packing house in Chiangmai and moved to laboratory before noon in the same day. Jiaogulan plants were harvested on June, August, and November, 2003, February, May, June 2004 and November 2005.

- Packaging Material. Tea bags were provided by The Royal Project Plant, Chiangmai, Thailand.

2. Equipments

- Tray dryer (Armfield Limited, England).
- Rotary vacuum microwave (March cool Ltd., Thailand).
- UV- VISIBLE Spectrophotometer (UV-1601 Shimadzu, Japan).
- Lyophilizer (Christalpa-4, Germany).
- Rotary evaporator (BuchiRotavapor R-205, Switzerland).
- HunterLab (ColorQUEST II, Hunter Associates Laboratory, Inc., Reston, Virginia, USA).
- Water activity (Novasina, Switzerland).
- Gas chromatography mass spectrophotometer (Voyager, PerSeptive Biosystems, Framingham, MA) with SPB 1701 column.
- Sep-Pak C₁₈ cartridge (Waters Corporation, MA).
- Vacuum oven (Squaroid, Lab-Line Instrument Inc., IL).
- Freeze dryer (25ES, Virtis Genesis, U.S.A.).

3. Chemicals

- The extract reagents: diethyl ether, n-butanol, ethanol and methanol (analytical grade, Merck, Germany).
- The HPLC reagents: methanol and acetonitrile (HPLC grade, Merck, Germany).
- ABTS (2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid diammonium salt) and Myoglobin (Sigma).
- The standard Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) (Aldrich Chem., Co.).
- The chemicals used for GC-MS analysis: Trimethylchlorosilane (Fluka Chemical Corp., USA), Trimethylsilylimidazole and N,N-bis-trimethylsilyl-trifluoroacetamide (Macherey-Nagel, Germany).
- Standard ginsenoside Rb1 (MP Biomedicals, LLC, U.S.A.).
- The commercial test kits: total antioxidant status (NX 2332), Cholesterol (CH200), LDL cholesterol (CH1350) and HDL cholesterol (CH203) (Randox Laboratory Ltd., U.K.).

Methods

This study was conducted in 4 phases:

Phase I: Raw material assessment

Phase II: Drying process of Jiaogulan

Phase III: Identification and effect of Jiaogulan extract in animal model

Phase IV: Product development of herbal beverage from Jiaogulan

1. Phase I: Raw Material Assessment

Raw material was fresh Jiaogulan plant (age over 5 months) from the Royal Pangda Station, Chiangmai. The plant was divided into 2 parts, leaf and stem, for the following main chemical analysis with two replications.

1.1 Total Solid and Moisture Content (A.O.A.C., 2000).

1.2 Total Antioxidant Activity (TAA) was measured by ABTS metmyoglobin method as Trolox Equivalent Antioxidant Capacity (TEAC) which modified from George and Irvine (1952); Miller *et al.* (1993); Miller and Rice-Evans (1997). The sample was extracted by ethanol with 200 rpm shaking at 25 °C for 6 hours. The dispersions were filtered with Whatman No 1 and evaporated by the vacuum rotary evaporator at 50 °C then lyophilized as the dried ethanolic extract. The TAA was measured using the metmyoglobin/ABTS spectrophotometric assay generating the chromogenic $ABTS^{\circ +}$ radical cat-ion from the interaction between ABTS (2,2'-azono-bis-(3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt) and Hydrogen peroxide. The mixed solution were metmyoglobin (76 μ M, 70 μ l), ABTS (5 μ M, 500 μ l), PBS (phosphate buffer saline, 5 mM, 980 μ l), hydrogen peroxide (500 μ M , 450 μ l) and sample solution 20 μ l froming a bluish-green complex. The reaction rate was recorded on time course program of UV-VIS spectrophotometer for 180 seconds at 414 mn. Trolox was used as an antioxidant standard using the calibration curve plotted with different amounts of Trolox as a standard curve. The TEAC of pure compounds as antioxidant in comparison to Trolox in %inhibition as following equation.

$$\%Inhibition = \frac{O.D.(Negative\ control) - O.D.(sample\ or\ standard)}{O.D. (Negative\ control)} \times 100$$

1.3 Saponin Assay was modified from Kwon *et al.* (2003); Wu *et al.* (2001). Jiaogulan was extracted by 80% (v/v) methanol with soxhlet method for 6 hours then filtrated with two layers of filter paper (Whatman No. 1). The filtrate was evaporated by rotary evaporator at 55°C, then lyophilized as the dried methanolic extract.

Determination of total crude saponin content was based on the methods of Kwon *et al.* (2003); Ando *et al.*(1971); Hong *et al.* (1979); the Korean Ginseng & Tobacco Research Institute (1991), the dried methanolic extract was

washed twice with 50 ml of diethyl ether to remove the fat content using a separatory funnel. The aqueous layer was extracted four times with 50 ml of water-saturated n-butanol. The butanol solution was washed twice with 30 ml of distilled water to remove the impurities. The 100 ml of remaining butanolic solution was transferred to a tarred round bottom flask, which was dried at 105°C to a constant weight (W_1), for the evaporation using a rotary evaporator under vacuum at 55°C. The flask with the evaporated residue was dried at 105 °C to a constant weight (W_2). Therefore, the difference in weight ($W_2 - W_1$) corresponded to the total crude saponin of the sample.

2. Phase II: Drying Process of Jiaogulan

2.1 Drying time of Jiaogulan

The fresh Jiaogulan was dehydrated by 2 drying methods; hot air drying and vacuum microwave drying; until the final moisture content went below 10%. The plant material was visually inspected for presence of foreign materials and then the leave were separated from the stems. The leaves were soaked in cleaned water to wash the dust and soil before the drying process. To obtain the drying time, the drying curve was illustrated by a simple diffusion model (Equation 1) by Maskan (2000).

$$MR = \frac{X - X_e}{X_0 - X_e} \quad (\text{Equation 1})$$

Which

MR = moisture ratio

X = moisture content (g water / g dry solid) at any time

X_e = equilibrium moisture content (g water / g dry solid)

X_0 = initial moisture content (g water / g dry solid)

For microwave drying, it can be assumed that $X_e = 0$ (Maskan, 2000).

2.1.1 The Hot Air Drying. The tray drier was operated at an air velocity of 0.5 m/s, parallel to the drying surface of the sample. Firstly, the drying curves were studied for each temperature to find the drying time. Moisture loss was recorded at 10 min interval during drying for the determination of dry curves by a digital balance. Jiaogulan, 100 g, was dried until equilibrium (no weight change). The temperature levels were 40, 50 and 60°C.

2.1.2 The Vacuum Microwave Drying. The rotary vacuum microwave (March cool Ltd., Thailand), which was a ϕ 650 x 950 mm. chamber and used 2,540 MHz microwave frequency, was used the vacuum condition (-700 mmHg.), 20 rpm rotary frequency and controlled by the digital control panel. Factor investigated in vacuum-microwave drying was microwave power intensity (1600, 2400, 3200 watt). Drying 500 g of Jiaogulan until the final moisture content was below 10%. Drying curve was constructed in order to determine drying time.

2.2 The Effect of Drying Process on Qualities of Dried Jiaogulan

Jiaogulan was dried by two drying methods; hot air drying and vacuum microwave drying, by drying time in 2.1. The assessments of dried Jiaogulan were as follow:

- Color L^* , a^* , b^* and ΔE by HunterLAB (ColorQUEST II, U.S.A.) using D65 illuminant, 10° observer.
- Water activity by thermoconstanter (Novasina, Swiss).
- Total solid and moisture content (A.O.A.C., 2000).
- Total antioxidant activity (TAA) by ABTS metmyoglobin method (George and Irvine, 1952; Miller *et.al.*, 1993).
- Total saponin (Kwon *et al.*, 2003).

2.3 Crop Variation

The experiment was conducted to evaluate crop variation of different seasons between crop 1 (August, 2003) and crop 2 (June, 2004). The processing treatments were using optimum microwave powers from 2.1. The total saponin content (Kwon *et al.*, 2003) and the antioxidant activity (George and Irvine, 1952; Miller *et al.*, 1993) were analyzed.

2.4 Statistical Analysis. Analysis of variance (ANOVA) was conducted to determine the effect of factors on drying parameters using SPSS[®] software. Tukey HSD test was performed to differentiate the significant effect.

3. **Phase III: Identification and Effect of Jiaogulan Extract in Animal Model**

3.1 Identification of Jiaogulan Extract

Extraction: Vacuum-microwave dried Jiaogulan was extracted by 3 methods, hot water, methanol and ethanol (Figure 11). The dried Jiaogulan was extracted with solvents at 1:30 proportions. The water extract method used double distilled water, heating in water bath shaker at 90°C for 10 min at 100 rpm. The methanol extract method used 80% methanol with Soxhlet extraction for 6 hours (Kwon *et al.*, 2003; The Korean Ginseng & Tobacco Research Institute, 1991). The ethanol extract method was conducted by shaking 95% ethanol at 200 rpm for 6 hours (Kawpinit, 1993; Poomecome, 1999; Maisutisakul and Pongsawatmanit, 2004). The extract was centrifuged at 1000 rpm for 5 min, then the supernatant was filtered (Whatman No. 1; filter paper). The filtrate was dried by rotary evaporator at 50°C, then lyophilized to the dried extract for chemical component identification. Gas Chromatography-Mass Spectrophotometry was used to characterize saponins in the Jiaogulan extract (Cui *et al.*, 1998; Cui *et al.*, 1999). A 40 mg extract sample and 5 ml of distilled water were mixed in a 15 ml test tube. The mixture was heated on a heating block for 30 min at 75°C and then supersonic extraction was used for 15 min prior to being centrifuged at 490 g for 5 min. The supernatant was purified by a Sep-

Pak C₁₈ cartridge. The column was washed with 10 ml of water followed by 10 ml of 30% methanol in water. The saponin fraction was eluted with 5 ml of methanol and collected in a 13 ml test tube with a Teflon cap. The solvent was evaporated at 65°C under a nitrogen stream. The dried fraction was dissolved in 3 ml of n-butanol and 75 mg of NaOCH₃. The alkaline cleavage reaction was carried out at 85 °C for 8 h. After washing with deionized water (2x1.2 ml), 600 µl of the butanol phase was evaporated to dryness at 80°C under a nitrogen stream. The residue containing the released aglycones was trimethylsilylated with 100 µl of a mixed reagent (BSTFA:TMSI:TMSCI = 3:3:2 v/v/v) at 70°C for 20 min and the derivatives were analyzed by GC-MS.

GC-MS Analysis. A GCQ (ThermoFinnigan) ion trap gas chromatography mass spectrophotometer (electron impact ionization, 70 eV) was used in this study. A DB1 column (column length 15 m., 0.25 mm I.D., film thickness 0.25 µm) and a SPB 1701 column (column length 15 m., 0.25 mm. I.D., film thickness 0.25 µm) were used for comparison. The column flow rate was 0.8 ml/min. The temperature program for the DB1 column was as follows: initial temperature was at 200°C for 0.1 min, which was increased at 10°C/min to 300°C, and finally to 320°C at a rate of 10°C/min with a hold for 10 min. The conditions for the SPB1701 column were 150°C for 0.1 min to 270°C at 10°C/min with a hold for 10 min. Injector temperature was 250°C, Ion source temperature was 200°C. The aglycones obtained from the samples were identified by comparing the retention time, relative retention time and the mass spectra obtained from the authentic saponin.

Ginsenoside Rb1 (Reference grade, MP Biomedicals, LLC, Ohio, U.S.A.) from ginseng (*Penax spp.*) was used as a reference standard. The standard (0.5 mg) was prepared in the same way as the sample. The reference substance was subjected to alkaline cleavage, derivatization and GC-MS analysis.

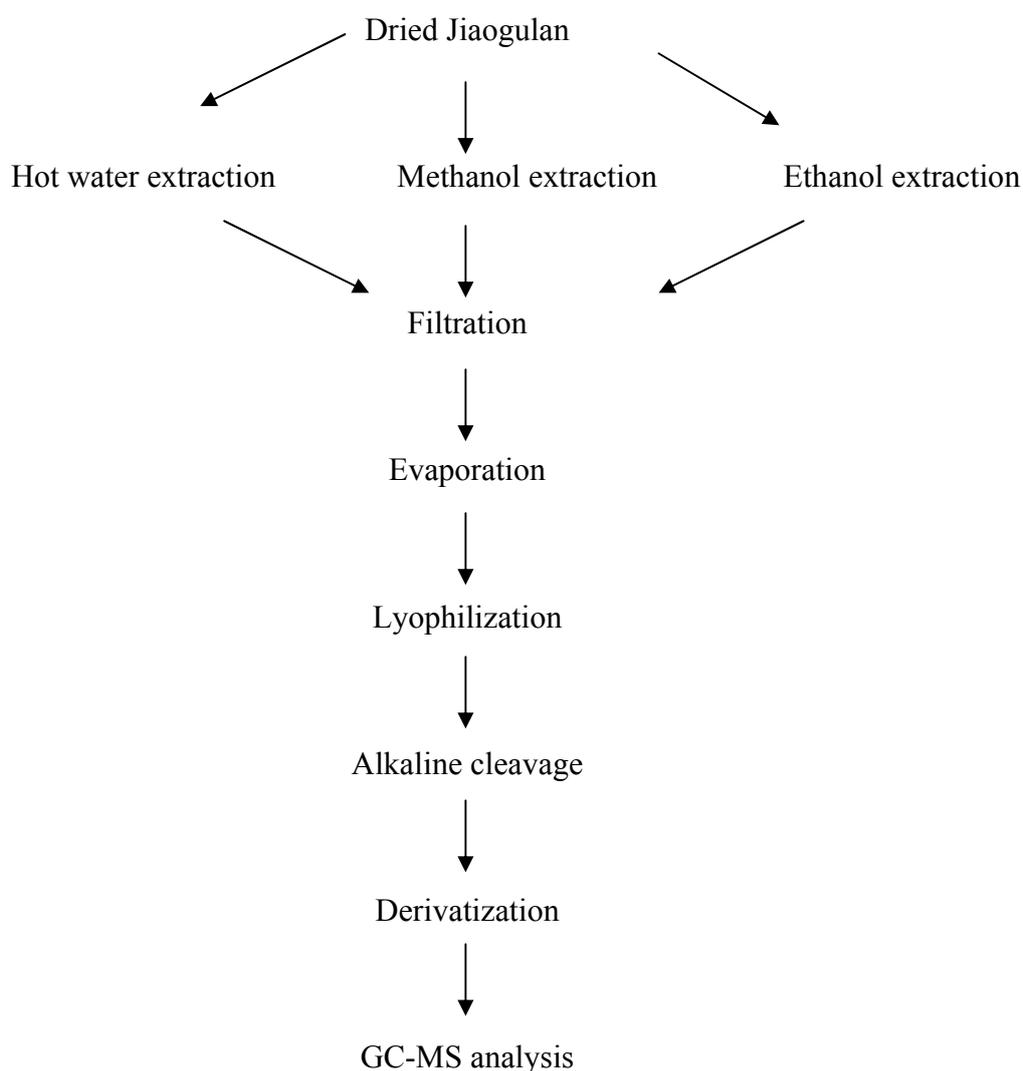


Figure 11 Identification of Jiaogulan extracts.

3.2 Toxicity Testing of Dried Jiaogulan

The acute toxicity test of Jiaogulan extract was investigated by the Medicinal Plant Research Institute, Department of Medical Sciences, Ministry of Public Health, Thailand. The methodology was followed by WHO (2000) and OECD (2001). Jiaogulan powder was refluxed twice with distilled water for 2 hours each. The Jiaogulan solution was evaporated until dry by the rotary evaporator with 39.82% yield. The dried extract was dissolved and distilled then the concentration was adjusted to 0.8 g/ml. The acute toxicity was investigated using ten mice (*Mus musculus* ICR strain); 5 male and 5 female. The Jiaogulan solution was fed to the

mice by oral administration , two time a day (morning and afternoon), with a dose of 16.0 g/kg body weight in 10 ml/kg amount. The experimental time was 14 days. There was a controlled group that was fed with distilled water in the same condition. At the end of the experiment period, the mice were anesthetized with ether and necropsied then their internal organs were visually observed.

3.3 Effect of Jiaogulan Water Extract on Serum Lipid in Animal Model

3.3.1 Experimental Animals and Diets

Ten female Sprague-Dawley rats (70 days old, mean body weights of 200 ± 10 g: Harlan Sprague-Dawley, Indianapolis, IN, U.S.A.) were housed in individual cages. Animal care was in compliance with applicable guidelines from the Purdue University policy on animal care and use. The diets and treatments are shown in Table 5. The rats were fed with a basal diet (DYET#101898, Dyets, Inc. Bethlehem, PA, U.S.A.) for 10 days and a high cholesterol diet (Table 4) for the next 2 weeks. During the 5th and 6th week, all rats were fed with a high cholesterol diet adding 3% water extract Jiaogulan (Figure 12). Fresh diets were prepared every 14 days and kept at -20 °C until feeding time. Food cups were refilled two times per week and feed consumption measured at these times. Body weights were recorded weekly.

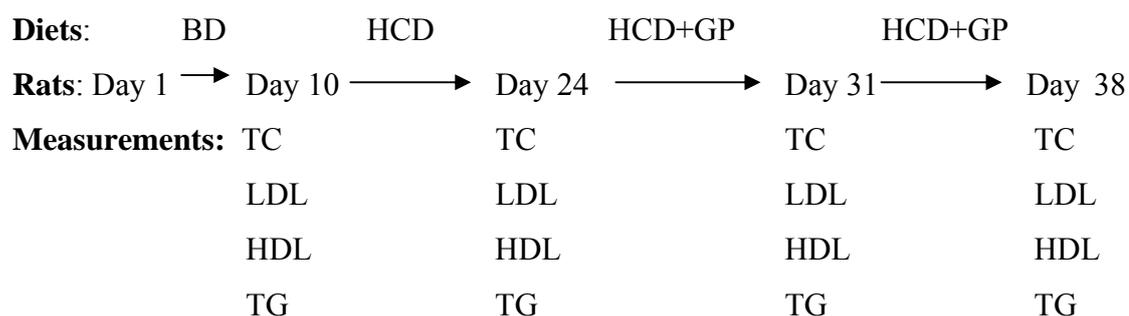


Figure 12 Experiment design for animal model study.

Note: BD=Basal diet, HCD=High cholesterol diet (1% cholesterol and 0.5% cholic acid), GP=3% Jiaogulan extract, TC=Total cholesterol level, LDL = Low density lipoprotein level, HDL = High density lipoprotein level, TG = Triacylglycerol level

Table 5 Compositions of the diets.

Ingredients	Diets		
	Basal diet	High Cholesterol Diet	High Cholesterol Diet + GP
Casein	200.00	200.00	200.00
L-Cystine	3.00	3.00	3.00
Cornstarch	367.078	352.078	322.078
DYETROSE	122.00	122.00	122.00
Sucrose	100.00	100.00	100.00
Soybean Oil	110.4	110.4	110.4
t-Butylhydroquinone	0.022	0.022	0.022
Cellulose	50.00	50.00	50.00
Mineral Mix#210025 ¹	35.00	35.00	35.00
Vitamin Mix#310025 ²	10.00	10.00	10.00
Choline Bitartrate	2.50	2.50	2.50
Cholic acid	-	5.00	5.00
Cholesterol	-	10.00	10.00
Water extract Jiaogulan	-	-	30.00

¹ Mineral mix # 210025 and ² Vitamin mix # 310025 are in Appendix 2

3.3.2 Sample Collections

Rat blood sample collections were performed on the 10th day, the 24th day, the 31th day and the 38th day of the feeding study. Blood (0.5-1.0 ml) was collected by retro-orbital sinus puncture from each rat (Hedirch and Bullock, 2004). Serum was isolated by centrifugation at 1200 g for 20 minutes at 4°C and the sample stored at -80°C until analyzed. After the dietary treatment period the rats were anesthetized by Ketamin-Xylezine injection and blood collected analyses. Blood was collected by cardiac puncture in anesthetized rats. All samples were kept on ice at the time of collection and frozen at -80°C until analyzed.

3.3.3 Serum Analysis

3.3.3.1 Total Cholesterol

Total cholesterol in serum was determined by a colorimetric method (RANDOX assay Kit Cat. No. CH 201, Antrim, United Kingdom). The assay principle is based on enzymatic hydrolysis and oxidation of cholesterol and the indicator compound, quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. The reagents consisted of 4-aminoantipyrine (0.03 mmol/l), phenol (6 mmol/l), peroxidase (≥ 0.5 U/ml), cholesterol esterase (> 0.15 U/ml), cholesterol oxidase (> 0.1 U/ml) and pipes buffer (80 mmol/L pH 6.8). The serum sample (10 μ l) was mixed with 1 ml of reagent, incubated at 37°C for 5 min, and absorbance measured at 500 nm against the reagent blank. The cholesterol standard was 5.17 mmol/l (200 mg/dl). The concentration of total cholesterol in the sample was calculated by

$$\text{Total cholesterol} = \Delta A_{\text{sample}} / \Delta A_{\text{standard}} \times \text{concentration of standard.}$$

3.3.3.2 Triacylglycerols

Serum triacylglycerols (TG) were determined by a colorimetric method (RANDOX assay kit Cat. No. TR 210, Antrim, United Kingdom). The assay principle is based on the enzymatic hydrolysis of TG with lipases and the indicator is a quinoneimine formed from hydrogen-peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic activity of peroxidase (Tietz, 1990). The enzyme reagent consisted of 4-aminophenazone (0.5 mmol/l), ATP (1.0 m.mol/l), lipases (≥ 150 U/ml), glycerol-kinase (≥ 0.4 U/ml), glycerol-3-phosphate oxidase (≥ 1.5 U/ml), peroxidase (≥ 0.5 u/ml). The serum sample (10 μ l) was mixed with 1000 μ l of enzyme reagent, incubated at 37°C for 5 min and absorbance measured at 500 nm against the reagent blank. The TG standard was 200 mg/dl (2.29 mmol/l). The concentration of TG in the serum was calculated by

$$\text{Total triacylglycerol} = \Delta A_{\text{sample}} / \Delta A_{\text{standard}} \times \text{concentration of standard.}$$

3.3.3.3 HDL Cholesterol Assay

Serum HDL cholesterol was determined by a colorimetric method (RANDOX assay Kit Cat. No. CH 203, Antrim, United Kingdom). The assay principle is based on the following: the low density lipoproteins (LDL and VLDL) and chylomicron fraction is precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL fraction, which remains in the supernatant, is determined. The precipitation reagents consisted of phosphotungstic acid (0.55 mmol/l) and magnesium chloride (25 mmol/l). The serum sample (200 μ l) was mixed with 500 μ l of precipitation reagent and centrifuged at 4000 rpm for 10 min. The supernatant (100 μ l) was mixed with reagent (CH 200 1 ml), incubated at 37°C for 5 min and absorbance measured at 500 nm against the reagent blank (Lopes-Virella *et al.*, 1977). The cholesterol standard was 200 mg/dL (5.17 mmol/l). The concentration of cholesterol in the supernatant was calculated as described above.

3.3.3.4 LDL Cholesterol

The LDL cholesterol was computed mathematically according to Friedewald's equation:

$$\text{LDL} = \text{TC} - \text{HDL} - \text{TG}/5 \quad (\text{Friedewald } et al., 1972).$$

3.3.4 Statistical Analysis

The data are expressed as mean \pm SD (n = 10). A paired Student's *t*-test was used to determine the significant differences from each time comparison between diets. Differences were considered statistically significant at $P \leq 0.05$ using the SPSS[®] software package (SPSS Inc., Chicago, IL).

4. Phase IV: Product Development of Herbal Beverage from Jiaogulan

4.1 Product Survey

The product survey of herbal beverage was conducted. The survey data were collected with the product type, brand, manufacturing, price and packaging. The locations were supermarket stores, department stores and some trade fair in Bangkok and Chiangmai Thailand. Duration time was between Novembers – December, 2003.

4.2 Focus Group Discussion

Focus groups discussion were conducted to investigate consumer perception and attitude of herbal beverage. Furthermore, the qualitative data was gathered for the product development. The method used was a focus group discussion with 8-10 participants who are Native Thai age over 35 years old. The discussion was done in 6 groups which were separated in 3 age ranges; 2 groups of participants 35 – 50 years old, 2 groups of participants 51 – 65 years old and 2 groups of

participants over 65 years old. The discussion was conducted by the trained moderator and the procedure was started with the introduction for 5 min, the reconnaissance for 20 min, in-depth investigation for 50 min and ending with the closure for 5 min (Galvez and Resurreccion, 1992; Resurreccion, 1998). The moderator guide is shown in Appendix 3. The qualitative data was collected by the observer and audio-tape and analysis process were also used (Casey and Krueger, 1994).

The main questions asked were:

1. What is the 'Herbal beverage' in your opinion?
2. What are your 3 favorite herbal beverages?
3. How often do you drink these herbal beverages?
4. Where do you buy herbal beverages?
5. What is your purpose for drinking herbal beverages?
6. What do you think about herbal beverages which can reduce fat and blood cholesterol?
7. What type of herbal beverages would you like to see on the market?
To develop the herbal beverage, what properties of herbal beverage do you want in term of physical and sensory factors?
8. What kind of packaging do you prefer for herbal beverages?
9. After testing the sample of herbal beverage, what is your opinion about color, odor, taste and others qualities?

4.3 Consumer Survey

The objectives of this survey were 1) to study the behavior, attitude, and perception of consumers to herbal beverages 2) to study the factors that effect consumer behavior and 3) to know consumer need for the development of herbal beverage from Jiaogulan. A questionnaire was developed from the focus group report. The questionnaire consisted of 3 parts; the consumer attitude and behavior of herbal beverage (37 questions), the development data of the herbal beverage sample (18 questions) and the personal data (5 questions) (Appendix 4). A three-point likert

scale was used to measure their attitudes toward herbal beverages. The scale categories corresponded to agree, neither agrees nor disagrees, and disagrees. The factor analysis using the seven-point rating scale was used to measure the importance of the factors. The scales categories corresponded to the most important, very important, important, neither important nor unimportant, unimportant, very unimportant and the most unimportant. The questionnaire was a pre-test by 20 consumers to ensure that the respondents understood the questionnaire.

The telephone survey with 40 consumers in 4 parts of Thailand demonstrated that 52.50% of respondents were interested in herbal beverages. So, the proportion of herbal beverage consumers to the population was $P = 0.525$. For a confidence interval of 95 percent, the standard error multiplied by 1.96 is the sampling error (Narins, 2002).

$$\text{Standard error} = 0.05 / 1.96 = 0.0255$$

The equation used for sample size calculation was as follow: (Aaker and George, 1983; Narins, 2002)

$$\begin{aligned} n &= P (1 - P) / \text{Standard error}^2 \\ n &= 0.525 (1 - 0.525) / (0.0255)^2 \\ &= 383.50 \end{aligned}$$

In conclusion, a sample size of 400 consumers was set for the survey. The consumer survey was conducted in 4 big cities in each parts of Thailand, the northern was Chiangmai (n = 100), the southern was Songka (n= 100), the north-eastern was Khonkane (n = 100) and the central part was Bangkok (n = 100). The surveys conducted at the canteen, public park, stadium, and the meeting room. The quota of sample depended on the following age range 25% were in the 35-45 age range, 25% 46-55 age range, 25% 56-65 age range and 25% were over 65 years old . The respondents were recruited by screening questions, *Have you ever drunk the herbal beverages?*, *Have you ever exercised?* and *Have you checked your health*

every year? Before the interview, the respondents were informed about the purposes of the study, then after the interview, each respondent was given a souvenir.

Sensory assessment: The sample was prepared by steeping dried Jiaogulan (5 g) in 90 °C water (250 ml) for 5 min, then filtering the beverage. The beverage sample was served in a 6 oz plastic cup covered with a slip and evaluated at 50 °C. The 9-point hedonic scale was used to measure product acceptance and preference and the scale categories corresponded to 9= *like extremely*, 8= *like very much*, 7= *like moderately*, 6= *like slightly*, 5= *neither like nor dislike*, 4= *dislike slightly*, 3= *dislike moderately*, 2= *dislike very much* and 1= *dislike extremely* (Jones *et al.*, 1955; Peryam and Pilgrim, 1957; Resurreccion, 1998). These values were assigned to the attribute of color, aroma, flavor and overall acceptability. Just-About-Right (JAR) scale were used to determine the intensity of the product attributes for developing direction and the scale categories corresponded to 1= *Much too little*, 2= *Somewhat too little*, 3= *just right*, 4= *Somewhat too much* and 5= *Much too much* (Vickers, 1988; Meilgaard *et al.*, 1999).

Data Analysis: The questionnaire was coded to facilitate data entry. The data were analyzed using the SPSS[®] version 11. The percentages of responses of the personal data and attitudinal data were calculated. Cross tabulations between age and acceptance, age and hedonic scale, age and product forms and location parts and acceptance were performed. Chi-square test was carried out to determine if the age, acceptance, hedonic scale and location significantly influenced the attitudinal data collected. The criteria of acceptance was 80% of total consumers. Multivariate analysis; factor analysis and Principal component analysis were used to study the factor effect to the consumer behavior. Logistic regression was carried out to determine the attributes effect to the acceptance.

4.4 Process Development of Jiaogulan Tea

4.4.1 Preparation of Jiaogulan Tea

The raw material of this research was the Jiaogulan leaves from the Tongrun station, Royal Project, Amphur Samern, Chiangmai. Jiaogulan leaves were dried by a vacuum microwave drier until the moisture content was below 7%, as specified by the Thai Industrial Standards Institute of green tea TIS 460-2526 (TISI, 1983). Dried Jiaogulan consisted of $6.94 \pm 0.13\%$ moisture content, $16.75 \pm 0.24\%$ total ash, $11.63 \pm 0.52\%$ insoluble ash, $5.11 \pm 0.30\%$ soluble ash and $49.87 \pm 0.57\%$ solid extraction yield by hot water. The dried Jiaogulan consisted of a 95.93 mg/g of total saponin. The dried Jiaogulan leaves were ground to fine particle by a milling process and sieved into 0.30–0.85 mm. particle size. The ground Jiaogulan leaves were then packed into tea bags of 1.0 g each (Figure 13).

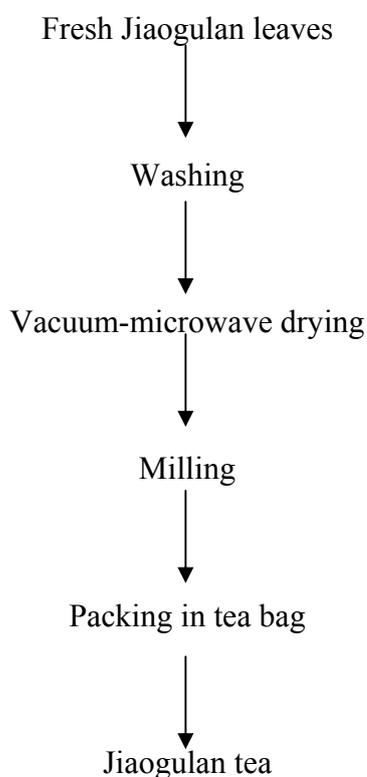


Figure 13 Jiaogulan tea process.

4.4.1 Determination of Kinetic for Jiaogulan Tea Infusion.

This study was using Jiaogulan tea bag per 50 ml of distilled water. The experiments were carried out in a water bath which was set to give a temperature 60, 70, 80, 90 °C inside the beaker with an accuracy of $\pm 1^\circ\text{C}$. Samples were taken at set intervals, 1, 2, 3, 4, 5, 10, 20, 30, 60 and 120 min.

Spiro and Jago (1982) showed the kinetic of tea infusion for loose tea, furthermore, Jaganyi and Price (1999); Jaganyi and Mdletshe (2000); Jaganyi and Ndlovu (2001) studied the infusion through the tea bag membrane in first order equation 2.

$$\ln (C_\infty / C_\infty - C) = k_{\text{obs}} t + a \quad (\text{Equation 2})$$

where C_∞ is the equilibrium concentration, C is the corrected concentration at time t , while k_{obs} is the observed rate constant and a is a semi empirical intercept.

The measurements were;

Solids extraction yield (SEY) was the infusion solid concentration which was determined by the drying method described by Liang and Bee (1992). SEY was calculated from the following equation (Liang and Xu, 2001; Liang and Xu, 2003):

$$\text{SEY (g/kg)} = \frac{\text{Solid concentration (g/ml) x Infusion volume (ml)}}{\text{Dry tea weight (kg)}}$$

The graph was plotted by SEY versus time, then fitted into the linear model of tea infusion kinetic to observe the constant rate of infusion.

4.4.2 Effect of Temperature and Time on Jiaogulan Tea Infusion

The kinetic tea infusion showed the time of infusion in different temperatures. This experiment was conducted by 3^2 factorial design with 2 factors; temperature (70, 80, 90°C) and time (1, 5, 10 min) with 2 replications. The response measurements were:

- Solid extraction yield
- Total crude saponin (Kwon *et al.*, 2003)
- Total antioxidant activity (George and Irvine, 1952; Miller *et al.*, 1993).

4.4.3 Statistical Analysis.

The data were analyzed by analysis of variance (ANOVA) with a second order model and the mean difference was determined by Tukey HSD using SPSS[®] software package (SPSS Inc., Chicago, IL).

4.5 Optimization of Saponin in Jiaogulan Tea.

This experiment was conducted to model the saponin dilution of Jiaogulan tea which was accepted by target consumers. The two-factor second-order rotatable design consisted of 6 design points called a pentagon design (Gacula and Singh, 1984). The independent variables were the saponin in dried Jiaogulan, X1 (0-500 mg) (calculated from dried Jiaogulan containing 95.93 mg/g of total saponin) and the water, X2 (50-250 ml). The experimental design of the coded and actual levels of variable is shown in Table 6. The responses included measuring the tea infusion; chemical properties, sensory descriptive and hedonic rating.

Table 6 The pentagon design with 6 design points of Jiaogulan tea treatments

Treatment	Saponin content (X1)			Water (X2)	
	Coded X1	Saponin	Dried Jiaogulan ¹ (g)	Coded X2	Water(ml)
1	1.0	500.0	5.21	0	150.0
2	0.309	327.3	3.41	0.951	245
3	-0.809	47.75	0.50	0.588	208.8
4	-0.809	47.75	0.50	-0.588	91.2
5	0.309	327.3	3.41	-0.951	54.9
6	0	250.0	2.61	0	150.0

¹ Saponin content was calculated from dried Jiaogulan containing 95.93 mg/g of total saponin.

4.5.1 Chemical Measurements

- Total antioxidant activity. TAA was analyzed by ABTS metmyoglobin method (George and Irvine, 1952 ; Miller *et al.*, 1993).

-. Total crude saponin (Kwon *et al.*, 2003).

4.5.2 Sensory Evaluation of Jiaogulan Tea Infusion

A hybrid descriptive analysis method (Einstein, 1991; Resurreccion, 1998) that was modified from both the Quantitative Descriptive Analysis (Tragon Corp., Redwood City, CA, U.S.A.) and the SpectrumTM Analysis methods (Sensory Spectrum, Inc., Chatham, NJ, U.S.A.) was used to evaluate Jiaogulan tea infusion. The selection of panels and Training for descriptive analysis are shown in Appendix 5. Twelve panelists were trained for 15 hours in one month. The trained panelists generated 13 attributes of Jiaogulan tea infusion (Table 7), and the consensus references with their intensity of each attribute (Table 8). Before evaluating samples in the booth, the panel was calibrated using reference standards and a warm-up sample to a reliability of rating. Each panel received 30 ml of

Jiaogulan tea infusion in 1 oz plastic cup with a cover slip. The temperature of the tea was controlled at 65-75°C (Resurreccion, 1998). Individually testing of samples was done with paper ballots (Appendix 6).

Table 7 Definition of descriptive attributes for Jiaogulan tea infusion

Attribute	Definition
Appearance	
Green yellow color	Light yellow to dark green
Clearness	The degree of clarity of sample
Sedimentation	The degree of sedimentary of sample
Aroma	
Dried leaf smell	Aromatic associated with dried grass or rice leaf or bamboo leaf
Green tea smell	Aromatic associated with green tea
Jiaogulan smell	Aromatic associated with Jiaogulan
Taste	
Sweet	The taste on the tongue associated with aqueous solution of sugar
Bitter	The taste on the tongue associated with aqueous solution of caffeine
Flavor	
Green tea flavor	Flavor associated with green tea
Jiaogulan flavor	Flavor associated with Jiaogulan
Feeling factor	
Astringency	The shrinking of the tongue surface caused by tannin or alum
Aftertaste	
Sweet	The sweet taste after product is swallowed
Bitter	The bitter taste after product is swallowed
Astringent	The shrinking of feeling after product is swallowed

Table 8 Reference samples for the 13 descriptive attributes of Jiaogulan tea infusion

Attribute	Reference	Intensity (mm)
Appearance		
Green yellow	0.05% tartarazine	45
	0.125 % Bromocresol green ¹	120
	Jiaogulan tea control ²	70
Clearness	Distilled Water	0
	0.4% Corn starch solution ³	150
	Jiaogulan tea control	47
Aroma		
Dried leaf aroma	Dried Jiaogulan	30
	Jiaogulan tea control	32.3
Green tea aroma	Green tea bag ⁴ (Japanese green tea, Chen cha)	15
	Jiaogulan tea control	40.5
Jiaogulan aroma	Jiaogulan tea control	65
Taste		
Sweet ⁵	2.0% sucrose	2
	5.0% sucrose	5
	10.0% sucrose	10
	16.0% sucrose	15
	Jiaogulan tea control	32.6
Bitter ⁶	0.05% caffeine	2
	0.08% caffeine	5
	0.15% caffeine	10
	Jiaogulan tea control	51.5
Flavor		
Jiaogulan flavor	Jiaogulan tea control	62.5
Green tea flavor	Green tea bag (Japanese green tea, Chen cha)	10
	Jiaogulan tea control	33.2

Table 8 (continued)

Attribute	Reference	Intensity (mm)
Feeling factor		
Astringency	0.07% Alum ⁷	27
	0.3% Alum	50
	tea bag / 1 hr soak ⁸ (Lipton green tea)	95
	Jiaogulan tea control	42
Aftertaste		
Sweet	2.0% sucrose	5
	5.0% sucrose	25
	10.0% sucrose	45
	16.0% sucrose	85
	Jiaogulan tea control	23.5
Bitter	0.05% caffeine	27
	0.08% caffeine	80
	Jiaogulan tea control	60.5
Astringency	0.07% Alum	5
	0.3% Alum	80
	tea bag / 1 hr soak (Lipton green tea)	110
	Jiaogulan tea control	59

¹ 0.125% Bromocersol green and 1% citric acid at pH = 4.0.

² Jiaogulan tea control consisted of Jiaogulan tea 1 g (total saponin 95 mg/g) in tea bag with extraction 80°C, 5 min.

³ Clearness reference was 0.04% corn starch (Yau and Huang, 2000).

⁴ Japanese green tea (Chen Cha) with extraction 90°C, 5 min.

⁵ Standard solutions for sweet were 2.0, 5.0, 10.0 and 16.0% sucrose solution (Meilgaard *et.al.* 1999).

⁶ Standard solutions for bitter were 0.05, 0.08 and 0.15% caffeine solution. (Meilgaard *et.al.*, 1999).

⁷ Standard solution for astringency was 0.07% Alum (Drobna, 2004).

⁸ Green tea bag (Lipton , green tea) soaked for 1 hour (Meilgaard *et al.*, 1990).

4.5.3 Hedonic Rating Test

Fifty consumers were members of the elder group of Thammasart hospital and the retired associations of the Air Force, Bangkok, Thailand. They were selected by the following criteria: age over 55 years old, health conscious and to engage in regular exercise. The Jiaogulan tea infusions were placed in a 2 oz plastic cup covered with a cap coded with three-digit random numbers and served monadically. Each consumer panel tested 6 samples, with a 10-15 min break after 3 samples test sessions. The order of serving to each panelist was randomized to minimize bias. Panelists rated each sample using a 9-point hedonic scale, with 1 being *dislike extremely* and 9 being *like extremely* (Peryam and Pilgrim, 1957) for the overall acceptability, color, odor, taste and aftertaste of the tea.

4.5.4 Statistical Analysis

The response data obtained from the chemical measurements, sensory descriptive analysis and consumer acceptance test were analyzed. A multiple regression analysis by stepwise regression was used to fit a second order model as follow:

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_{12} X_1 X_2 + b_{11} X_1^2 + b_{22} X_2^2$$

Where Y was the response variable (physicochemical, sensory descriptive and consumer acceptance data, X_1 was total crude saponin content in dried Jiaogulan and X_2 was water for tea dilution. The coefficients of the polynomial were presented by b_0 (constant term), b_1 and b_2 (linear effects), b_{11} and b_{22} (quadratic effects) and b_{12} (interaction effects) (Hu, 1999).

The response surface graphs from the quadratic model were generated by a design-expert program (Design-Expert version 6.0.10, Stat-Ease Inc., MN). Finally, all graphs were optimized by using the criteria: (1) consumer acceptance score > 6.0. (Resurreccion, 1998; Grosso and Resurreccion, 2002) and (2)

maximum saponin content and total antioxidant activity. An optimized formula of saponin content of Jiaogulan tea was figured out for raw material which has different saponin content.

4.6 Quality Measurement of Finished Jiaogulan Tea Product

The finished Jiaogulan tea product was analyzed for chemical, physical and microbial quality. The chemical analysis were the moisture content, total solid, total ash, insoluble ash, soluble ash, extract solid yield, protein, fat, crude fiber (AOAC, 2000), total saponin (Kwon *et al.*, 2003), and total antioxidant activity (George and Irvine, 1952; Miller *et al.*, 1993). The physical analysis is the color measurement (L^* , a^* , b^*). The microbial analysis included total aerobic microbial count, yeast and moulds, *Escherichia coli*, *Staphylococcus aureus*, *Clostridium spp.* and *Salmonella spp* (AOAC, 2000).

4.7 Consumer Acceptance Test of Jiaogulang Tea Infusion

The objectives of this consumer test were to evaluate the acceptability of Jiaogulan tea and to access the effect of health information on the buying decision of consumers. Two hundred consumers who lived in Bangkok (n=100) and Chiangmai (n=100) participated in this study. The surveys conducted at the sport centre, the golf club, the canteen, and the public park. Threes criteria were selected consumer screening: over 55 years old, exercise at least once a week and yearly medical checkups. Before the interview, the respondents were informed about the purposes of the study. The method used was semi-home use test that allowed the participants to brew the tea sample by themselves. Each consumer received a Jiaogulan tea bag and a cup of hot water. The Jiaogulan tea sample, contained saponin 161.68 ± 5.57 mg/g, was prepared in a tea bag, 1.80 g / bag for the optimum condition (292 mg of saponin per tea bag). The following brewing direction was given to the panelists, pour the hot water (90°C) in a cup (4.5 oz size) then immerse the tea bag for 10 min before testing. The questionnaire consisted of 3 parts, the demographic information, the acceptability test and the product information parts

(Appendix 7). Panelists rated Jiaogulan tea using a 9-point hedonic scale (Resurreccion, 1998; Meilgaard *et al.*, 1999), with 1= *dislike extremely*, 5= *nether like nor dislike* and 9=*like extremely* (Peryam and Pilgrim, 1957) for overall liking, color, odor, overall flavor, sweetness, bitterness and aftertaste acceptance. After the consumer test, the information was provided to consumers. The product research information described to the consumers was that Jiaogulan tea consisted of antioxidant and saponins which are the same compound as ginsengoside Rb1 and Rg1 in ginseng. Additionally, in the animal study, it was found that Jiaogulan tea extract could reduce triglyceride and cholesterol level in rat serum within 2 weeks. Then, consumers were asked about their buying decisions. Each respondent was given a souvenir after finish testing.

The mean values and standard deviation of consumer acceptance data were obtained using the SPSS[®] software package (SPSS Inc., Chicago, IL). The discriminant analysis technique was used to predict buying decision of consumers. The McNemar test was performed to determine significant differences between before and after buying decisions.

4.8 Effect of Storage Temperature on Stability of Saponin and Antioxidant Activity

This study was conducted to examine the stability of saponin content and antioxidant activity during the storage time of Jiaogulan tea.

4.8.1 Sample Preparation

Fresh Jiaogulan was harvested from the Rayal Tongrun station at Amphur Samern. Jiaogulan leaves were dried by the vacuum microwave drier for 25 min at 2400 watt and ground by pin mill into fine particle. Then, dried Jiaogulan was vacuum-packed in aluminum foil and stored at -20°C until used. The tea prepared by packing 1 gram of dried Jiaogulan in a tea bag and sealed by a sealer

machine. The Jiaogulan tea products were prepared in 2 replications and samples were stored under designated conditions.

4.8.2 Storage Condition

Samples were stored at 3 storage conditions at 23-25 °C (ambient temperature at Purdue University, Indiana), 35°C and 45°C (accelerated condition). These conditions were suitable for dried food product (Labuza and Schmidl, 1985). The sampling time was on 0, 1, 2, 3 and 4 months for antioxidant activity analysis and total saponin analysis.

4.8.3 Total Saponin Analysis (Kwon *et al.*, 2003)

The total saponin assay was modified from The Korea Ginseng & Tobacco Research Institute (1991) and Kwon *et al.* (2003).

4.8.4 Total Antioxidant Activity.

Total antioxidant activity was determined by the Total Antioxidant Status kits (NX 2332, Randox Laboratories Ltd., U.K). Miller *et al.* (1993) described the principle that ABTS[®] (2,2'-azino-di-[3-ethylbenzthiazoline sulphonate]) was incubated with a peroxidase (metmyoglobin) and H₂O₂ to produce the radical cation ABTS^{®+} which had a relatively stable blue-green color at 600 nm. TAS kits consisted of 3 reagents; buffer (phosphate buffered saline 80 mmol/l, pH 7.4), chromogen (metmyoglobin 6.1 µmol/l and ABTS[®] 610 µmol/l), substrate (hydrogen peroxide in stabilized form 250 µmol/l) and standard (Trolox, 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid, lot No. 175NX = 1.71 mmol/l). The assay procedure started with reagent blank solution using 20 µl of double distilled water, standard solution 20 µl and sample 20 µl. All solution were mixed with chromogen 1 ml then incubated to bring the temperature to 37 °C, then read the initial absorbance (A₁) was read at 600 nm. After that, the substrate 200 µl was added to

start the kinetic reaction time. The absorbance was read again in exactly 3 minute (A_2). The ΔA was from $A_2 - A_1$. The antioxidant status was calculated from;

$$\text{Factor} = \text{concentration of standard} / (\Delta A \text{ blank} - \Delta A \text{ standard})$$

$$\text{TAS mmol/l} = \text{Factor} \times (\Delta A \text{ Blank} - \Delta A \text{ Sample})$$

4.9 Construct Product Specification of Jiaogulan Tea

The product specification was established from the product description, raw material specification, process control, quality control and packaging specification.

4.10 Cost of Jiaogulan Tea Product

Cost of Jiaogulan tea product was calculated from the raw material cost, packaging cost and cost accounting.

RESULTS AND DISCUSSION

1. Phase I: Raw Material Assessment

1.1 Raw Material Assessment

The fresh Jiaogulan plant as raw material was harvested in the morning from the Royal Pangda station, Samern, Chiangmai. The aerial parts of Jiaogulan were separated into 3 parts; top, leaf and stem. The moisture content of all parts was approximately 81.55- 83.91% and the total solid was 16.09 – 18.86% (Table 9). The total solid of the leaf was significantly higher ($p \leq 0.05$) than the stem, but non significantly different from the top. This means that, the leaf part consisted of more solid content than the stem because it contains many substances such as nutrients and minerals which are supplied to the plant. The yield was examined by calculation to percent compared to total weight and all parts were significantly different ($p \leq 0.05$). As a result, the leaf provided the highest yield, 62.95%, and the stem had yield, 33.57%, but the top had the lowest yield, 3.48% (less than 5% of total weight). Therefore, the top part was not suitable for the processing because of fewer yields, so this part was gathered with the leaf part. The leaf and stem parts were selected for future study.

Table 9 The yield, moisture content and total solid of Jiaogulan parts

Items	Jiaogulan parts		
	Leaf	Stem	Tumip top
Moisture content (%)	81.55±0.58 ^b	83.82±1.32 ^a	83.91±4.45 ^b
Total solid (%)	18.45±0.58 ^a	16.18±1.32 ^b	16.09±4.45 ^a
Yield (%) ¹	62.95±6.45 ^a	33.57±7.25 ^b	3.48±1.02 ^c

¹ % Yield was done with 4 replications.

The different letters in the same row are significantly different at $p \leq 0.05$.

1.2 The Antioxidant Activity

The measurement of total antioxidant activity, ABTS/H₂O₂ / metmyoglobin method, derives from the principle that metmyoglobin makes hydrogen peroxide, the relative long-lived radical, solutes in water and forms the reactive oxygen. The reactive oxygen will oxidize ABTS (2,2' azono-bis-(3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt) to ABTS^o radicle. This oxidation makes blue-green color by oxidized ABTS which absorbed peak at 414 nm. (Gorge and Irvine, 1952). Trolox, antioxidant standard from stable tocopherol, in different concentrations can inhibit production of oxidized ABTS, reducing the development of blue-green color at absorbance 414 nm. The antioxidant activity of Jiaogulan leaf and stem shows in Figure 14 from Trolox standard curve (Appendix 1). The leaf contains TEAC 23.04 mg Trolox/ g, whereas the stem contains 5.26 mg Trolox/g. Vinson *et al.* (1998) reported that green leafy vegetables such as spinach, were able to enrich the lipoproteins by binding with them and subsequently protecting them from oxidation. But leafy spices like thyme, marjoram, basil, sage, and summer savory all showed pro-oxidative activity for foods exposed to light, while the same food stored in the dark confirmed the antioxidative effect of the spices. The effect of photosensitization of chlorophyll present in spices may be more important than the effect of the antioxidants for food exposed to light (Yanishlieva-Maslarova and Heinonen, 2001). The antioxidant activity of the Jiaogulan plant in this study indicated that the antioxidant in fresh leaf was approximately five time higher than one in the stem.

1.3 Total Saponin

Total saponin in Jiaogulan was analyzed by analytical methods of ginseng component (Korea Ginseng & Tobacco Research Institute, 1991). Since, The Medicinal Plant Research Institute (2005) concluded that four of the same saponins in Jiaogulan and ginseng are ginsenoside Rb₁ (gypenoside III or gypnsaponin C), ginsenoside Rb₃ (gypenoside IV), ginsenoside Rd (gypenoside VIII) and ginsenoside F₃ (gypenoside XII). Hence, the total saponin content in Jiaogulan could be analyzed

by the same method as the ginseng analytical method. Total saponin content in Jiaogulan parts show in the Figure 15. The total crude saponin in leaf was at 204.07 mg/g, whereas the stem contained only 105.19 mg/g saponin. This results showed that the saponin in fresh leaf was approximately two time higher than one in the stem.

In conclusion, the leaf contained higher antioxidant activity and total saponin than the stem. Therefore, it has more potential to be a raw material for Jiaogulan tea.

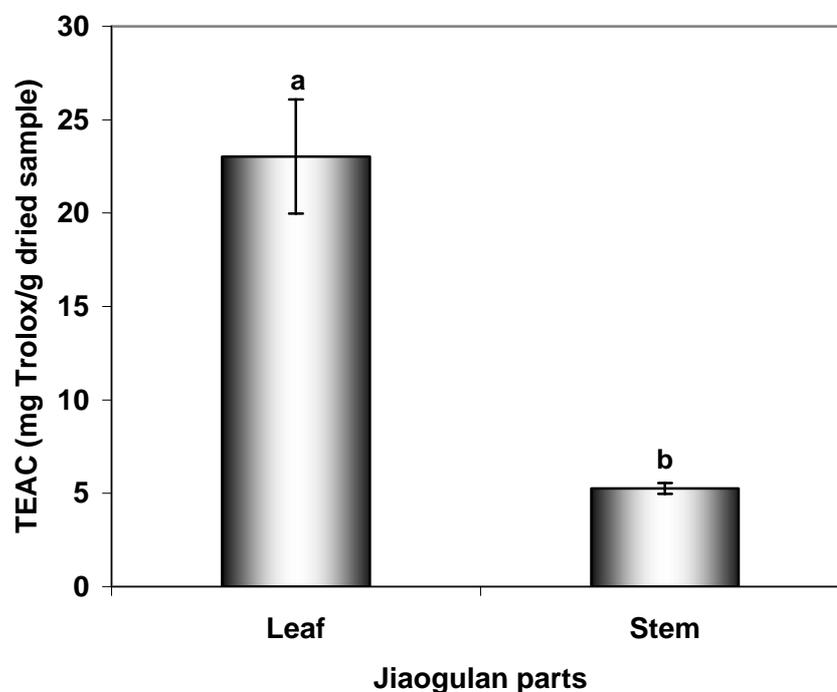


Figure 14 The trolox equivalent antioxidant capacity of leaf and stem of fresh Jiaogulan.

Note: Data present mean \pm S.D. (n=3).

The different letters are significantly different at $p \leq 0.05$.

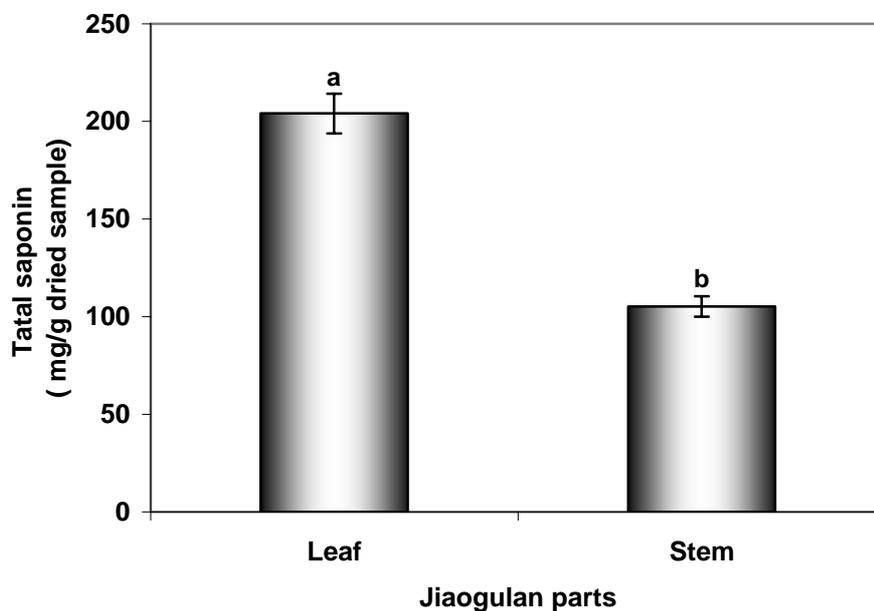


Figure 15 The total saponin in leaf and stem of fresh Jiaogulan.

Note: Data present mean \pm S.D. (n=3).

The different letters are significantly different at $p \leq 0.05$.

2. Phase II: Drying Process of Jiaogulan

2.1 Drying Time of Jiaogulan

2.1.1 Hot Air Drying

The moisture content; in term of MR (moisture ratio); versus time curves for hot air drying of Jiaogulan with different temperature was shown in the drying curve (Figure 16). The graph shows that higher temperatures had more water content reduction. Drying at 60°C the moisture was lost faster than at 50 and 40°C. Fellows (1996) explained that initially the moisture decreased rapidly because the water moved from the interior at the same rate as it evaporated from the surface which was in a constant-rate period. The factors were dry-bulb temperature, relative humidity, and air velocity. The drying rate remained constant until a critical moisture

content was reached, after that it declined until it reached the equilibrium, called the falling-rate period. The drying rate was calculated at different times and plotted against average moisture content as shown in Figure 17. A constant rate period was not observed in hot air drying of Jiaogulan because the entire drying process for the samples occurred in the range of a falling rate period in this study which was in agreement with hot air drying banana (Maskan, 2000). It has been reported that almost all of the drying of biological products takes place in the falling rate period (Madamba *et al.*, 1996). For drying on 100 g of fresh Jiaogulan, the drying times to reach about the 10% moisture content (0.1 g water/g dry solid) were 480 min (8 hours) at 40°C, which was the longest time. On the other hand, drying at 60°C took the shortest time about 210 min (3.5 hours). And using 50°C as the drying temperature, with the same fixed condition, air velocity 0.5 m/s, the drying time was 300 min (5 hours). So, this result supported the theory that a higher drying temperature provided a higher drying rate of dehydration (Devahastin, 2000).

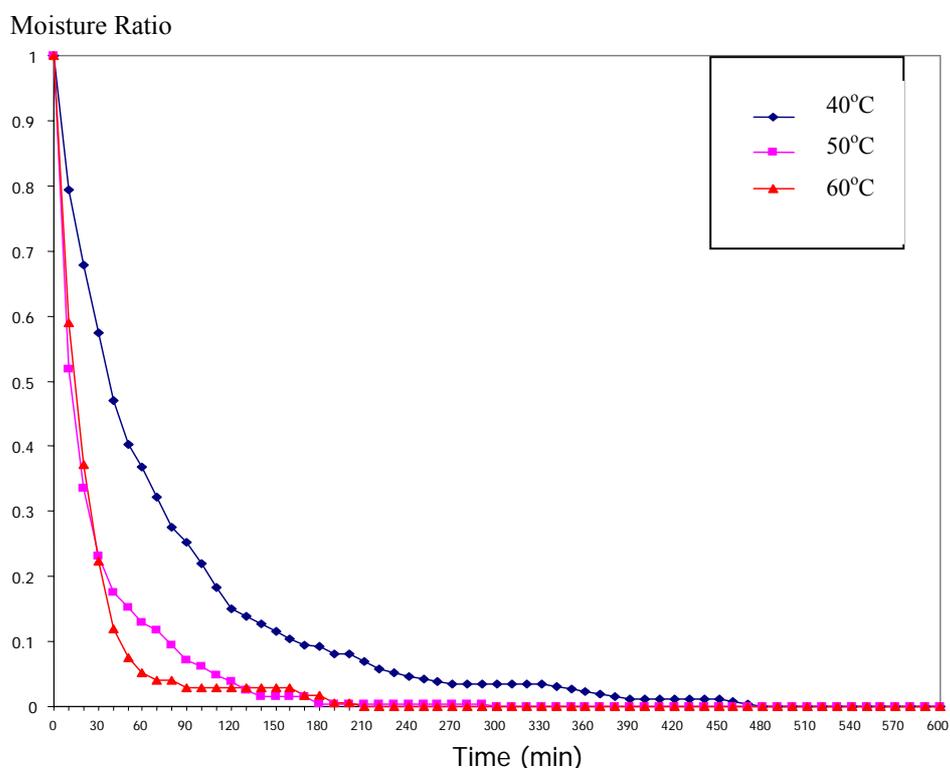


Figure 16 Drying curve of Jiaogulan by tray drying with the different temperatures.

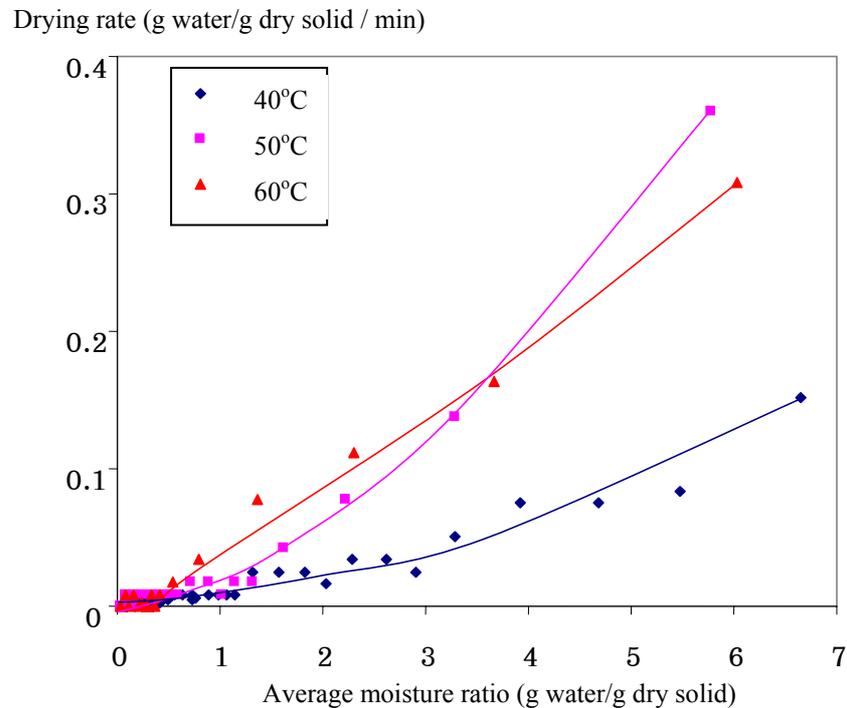


Figure 17 Drying rate of Jiaogulan dried by tray drier.

2.1.2 Microwave-Vacuum Drying

In this study, the drying curve of dried Jiaogulan by vacuum microwave is shown in Figure 18. The microwave drying with a 1600 watt take a longer time than using 2400 and 3200 watt. According to the drying rate, it was lower than using higher power (Figure 19). As illustrated, microwave-vacuum drying was a much faster drying process than hot air drying. The microwave phenomenon greatly reduces the time required for complete drying by more than 30% when compared to the conventional method (Drouzas and Schubert, 1996). The results were in agreement with previous studies such as Lin *et al.* (1998) using dried carrot slices and Maskan (2000) using dried bananas. Microwave-vacuum drying used low temperatures and fast mass transfer conferred by vacuum (Yongsawatdigul and

Gunasekaran, 1996) combined with rapid energy transfer by microwave heating, generates very rapid, low temperature drying. The total drying times required for reaching final moisture content of about 0.1 g water/ g dry solids were 40, 25 and 17 min at 1600, 2400 and 3200 watt, respectively. The results indicated that mass transfer within the Jiaogulan leaves was rapid during microwave heating because heat was generated within the leaves, creating a large vapor pressure differential between the center and the surface of the leave (Lin *et al.*, 1998). Moreover, the rotary system of this microwave made faster dehydration possible because it provided heating to all surfaces of the leaves. Kudra and Mujumdar (2002) showed that the performance of microwave drying was better than that of convective drying, as well as time saving (Figure 9). In addition, a volumetric heat transfer mechanism coupled with drying in the absence of oxygen (vacuum) provides an ideal low-temperature drying technique.

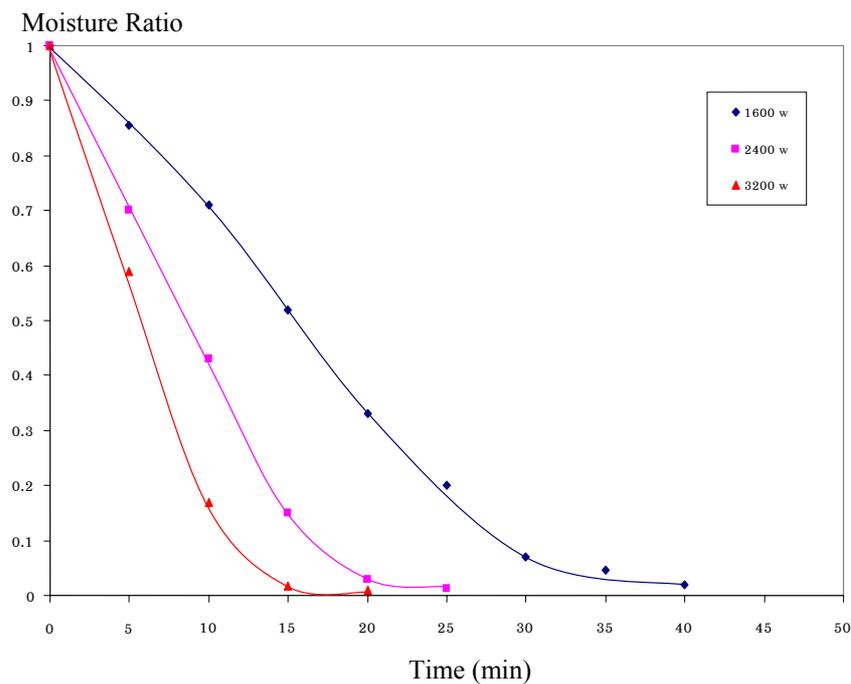


Figure 18 Drying curve of Jiaogulan dried by vacuum microwave drier with the different microwave power levels.

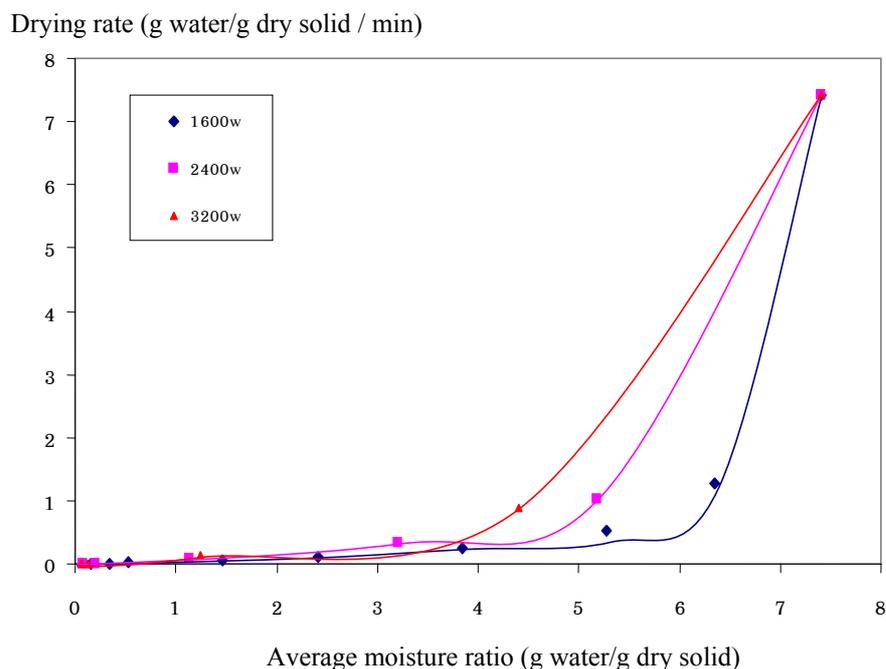


Figure19 Drying rate of Jiaogulan dried by vacuum microwave.

2.2 The Effect of Drying Process on Qualities of Dried Jiaogulan

2.2.1 Physical Properties

Mean values of moisture content shows that the moisture content of dried Jiaogulan was 10% or less than 10% (Table 10). The water activity of microwave dried Jiaogulan was less than 0.55 that is the lower limit for life to continue (Fellow, 1996), meanwhile, bacteria, mold and yeast cannot survive in this condition. And the yield of hot air drying and vacuum-microwave drying were approximately 8.20 – 13.00%. During air drying, liquid diffuses to the surface of food product from the interior and carries solutes with it (Fellows, 1996). As the surface moisture evaporates, solutes concentrate and precipitate, leaving a hard and dry skin. While vacuum-microwave drying as heat was generated within the product, resulting *in situ* vaporization of water which was able to rapidly diffuse out of the

tissue, without carrying dissolved solutes with it (Lin *et al.*, 1998). So, vacuum-microwave drying was more efficient than hot air drying.

The color is an important parameter of dried herbs. As illustrated in Table 9, the higher temperature of tray drying produced darker color (lower L* value), in contrast, the higher microwave power produced brighter color (higher L* value). This was the same results of yellowness (b* value). On the other hand, the green color shown in a* value, which trail of more greenness in microwave drying than hot air drying. For color difference were in ΔE . Lin *et al.* (1998) examined the color property of vacuum microwave, air and freeze dried carrot slices. The results showed that vacuum microwave drying produced darker colored products than freeze drying but significantly lighter colored products than hot air drying. The L* and b* value of vacuum microwave drying were significantly higher than hot air drying. All these results indicated drying with vacuum microwave provided better color quality of Jiaogulan leaves than hot air drying.

Table 10 Physical properties of dried Jiaogulan by hot air drying and microwave vacuum drying

Treatment	Moisture (%)	Yield (%)	a _w	L*	a*	b*	ΔE
Hot air drying ¹							
40°C / 480 min	10.65	13.00	0.641	42.05	-0.48	6.49	56.51
50°C / 300 min	6.48	10.00	0.426	41.65	-1.20	6.22	56.78
60°C / 210 min	5.95	11.00	0.428	41.35	-0.89	5.91	57.03
Microwave drying ²							
1600 watt/40 min	8.95	9.40	0.498	40.94	-0.61	6.03	57.41
2400 watt/25 min	9.67	9.70	0.551	42.49	-0.72	7.77	56.68
3200 watt/17 min	6.63	8.20	0.450	43.02	-2.14	8.57	56.15

¹ Hot air drying by tray drier with 100 g of raw material

² Microwave drying by vacuum microwave dried with 500 g of raw material

2.2.2 Total Saponin Content

The total saponin contents of hot air dried and vacuum-microwave dried Jiaogulan were not significantly different (Figure 20). The highest crude saponin produced 134.16 mg/g dried Jiaogulan by 2400 watt with vacuum-microwave drying, followed by hot air drying at 129.25 mg/g but the lowest was vacuum-microwave drying with 3200 watt. The saponin compound was a heat resistant substance. Bobeyko and Kintia (1996) studied the thermal behavior of steroidal glycosides; furostanol, spirostanol and spirosolane class and their sapogenins. They found that steroidal glycosides and sapogenins were thermostable up to 150-170°C. Within the temperature range of 190-250°C the carbohydrate moiety of aglycone C-3 was fragmented, and at more than 270°C saponins were destroyed. This result of saponin content in Jiaogulan was compared to the Korean Food Standard Code (MHW, 2002) which extracted saponin from ginseng. The saponin contents were more than 80 mg/g of the extract. Kwon *et al.* (2003) initiated the extraction of ginseng saponins by a microwave-assisted process (MAPTM) which can reduce the extraction time from 12 hours to a few seconds by using 300 watt for 30 second and yield, 5.31% of saponin. Therefore, the vacuum-microwave drying could reduce the process time of Jiaogulan drying.

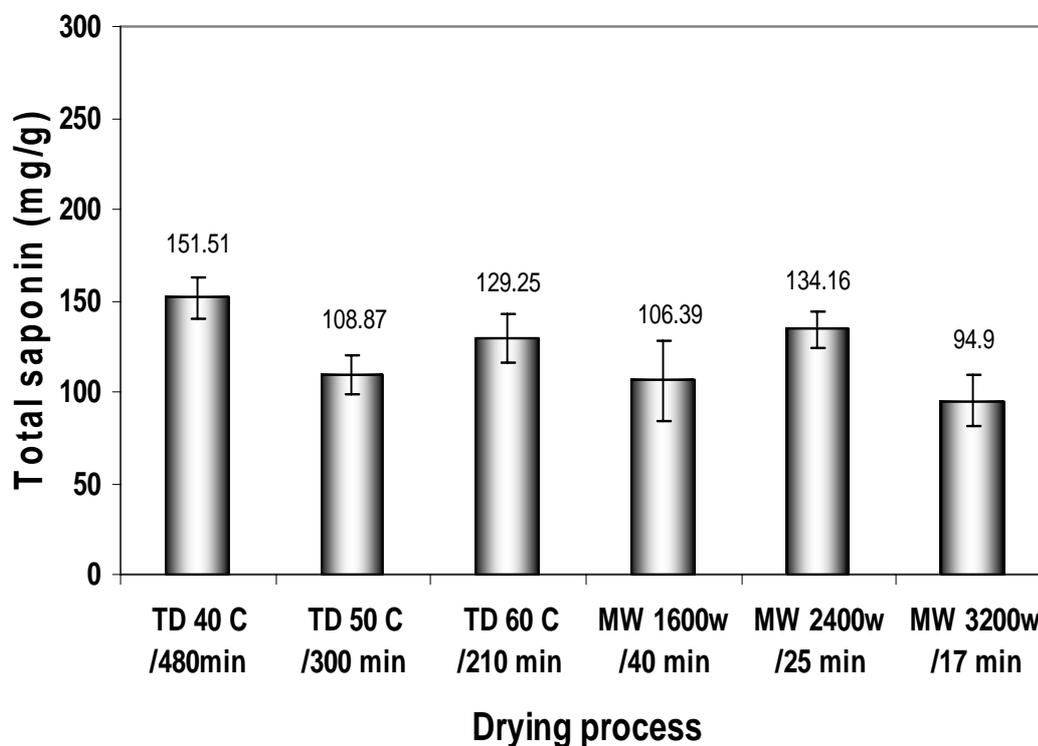


Figure 20 Total saponin in dried Jiaogulan dried Jiaogulan using hot air drying and vacuum-microwave drying.

Note: Data are presented as mean value \pm SD (n=2).

2.2.3 Antioxidant Activity

The antioxidant activity of Jiaogulan leaves drying by tray drier and vacuum-microwave is shown in Figure 21. There are significantly higher TEAC in Jiaogulan by vacuum-microwave drying, 37.98 – 56.11 mg trolox / g, whereas Jiaogulan using hot air drying consisted of TEAC 4.85 – 7.70 mg trolox / g. Pokorny and Schmidt (2001) stated that during the drying process, lipid oxidation was restricted because of short drying time (only a few minutes or still less in modern drying equipment), and the presence of water vapour in the atmosphere. Antioxidants are usually not damaged during drying, and their evaporation is only moderate. Nido *et al.* (2003) reported that optimum microwave power can retain total antioxidant activity more than tray drying for asparagus. Hence, vacuum-microwave drying is a

very rapid drying process and requires only minutes instead of hours or days of drying time. It has recently been shown that thermal processing of sweet corn caused antioxidant activity and total phenolics to increase by 44% and 54%, respectively (Dewanto *et al.*, 2002). Other related studies on thermally processed tomatoes, tomato juice and other products have shown that heating caused an increase in their overall antioxidant potential due to production of nonnutrient antioxidants (Nicoli *et al.*, 1997; Anese *et al.*, 1999; Dewanto *et al.*, 2002). Nicoli *et al.* (1997) suggested that although natural antioxidants are lost during heating, the overall antioxidant properties of foods could be maintained or enhanced by the development of new antioxidants. Pokorny and Schmidt (2001) stated that antioxidant properties might increase resistance against oxidation, transform antioxidants into more active compounds, such as glycosides into aglycones. Accordingly, saponin glycosides in Jiaogulan changed into aglycones in processing and storage.

In conclusion, the better process was vacuum-microwave drying because it could remain more total antioxidant activity than hot air drying. Finally, the optimum process was microwave drying at 2400 watt for 25 min, which retained more antioxidant activity and took less time to process.

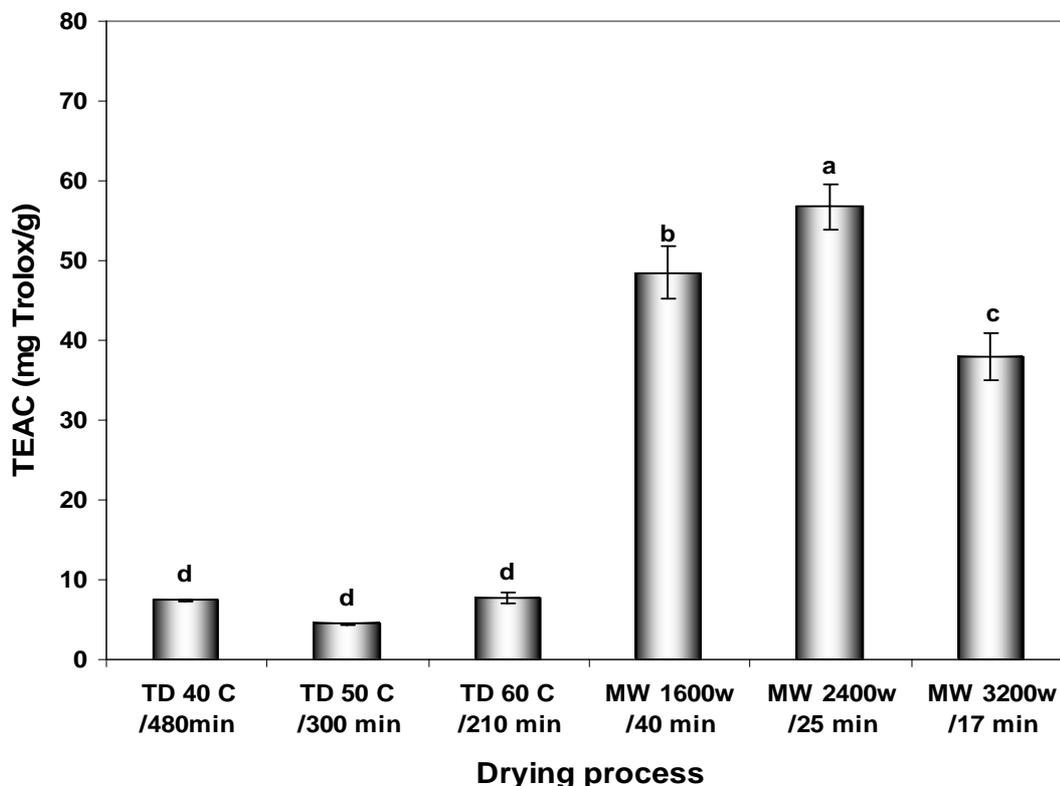


Figure 21 The trolox equivalent of antioxidant capacity of dried Jiaogulan using hot air drying and vacuum-microwave drying.

Note: Data are presented as mean value \pm SD (n=2).

The different letters are significantly different by one-way ANOVA and Turkey's student range test ($p \leq 0.05$).

2.3 The Crop variation

This experiment was conducted to evaluate the crop variation in different seasons between crop 1 (August, 2003) and crop 2 (June, 2004). The total saponin contents vary significantly between crops ($p \leq 0.05$) (Table 11). The crop variation was caused by the season and climate of cultivation (Hartmann *et al*, 1988; Basra, 1998). Crop 2 which grew in the summer time, had a higher saponin content than crop 1, which grew in the rainy season. In summer time, the plant received less water, so the substances in the plant were high in concentration. Another reason for the variation was that the moisture in the plant was evaporated by the hot weather that

produced a more concentrated substance in the plant. It had been observed that the saponin composition in soybean seeds is not affected by a difference in cultivation conditions but it is peculiar to variety (Shiraiwa *et al.*, 1991; Tsukamoto *et al.*, 1995). The antioxidant activity were significantly different between crops (Table 11). Masen and Bertelsen (1995) pointed that antioxidant activity varied according to the country in which the plant was grown. Because some phenolic components, which had antioxidant activity, may degrade at higher temperature (Moure *et al.*, 2001).

Table 11 Chemical properties of dried Jiaogulan by microwave-vacuum drying¹ of two crops

	Crop 1 (August, 2003)	Crop 2 (June, 2004)
Total saponin (mg/g)	134.16 ^b	186.39 ^a
TEAC (mg Trolox/g)	56.72 ^a	38.96 ^b

¹ Microwave-vacuum drying using 2400 watt for 25 min

The different letters in the same row are significantly different ($p \leq 0.05$).

3. Phase III: Identification and Effect of Jiaogulan Extract in Animal Model

3.1 Identification of Jiaogulan Extract

The preparation method for identification was alkaline cleavage and derivatization of Jiaogulan extract. Cui (1995) reported that alkaline cleavage of ginsenosides, followed by trimethylsilyl (TMS) derivatization and gas chromatographic-mass spectrometric analysis was a suitable method for analysis and authentication of *Panax* drugs. Compared to acidic cleavage, alkaline cleavage did not produce as many artifacts (Cui *et al.*, 1993). The preparation of Jiaogulan extract used alkaline cleavage, followed by TMS derivatization and analysis by GC-MS. The GC-MS chromatograms of 3 Jiaogulan extracts; water extract, methanol extract and ethanol extract and reference standard (ginsenoside Rb1), as determined by SPB 1701 columns are shown in Figure 20-25. Three peaks are determined in Jiaogulan methanol and ethanol extract but the water extract for Jiaogulan presents only two peaks.

The mass spectra of three aglycone compounds found in the Jiaogulan extracts are as follow (Figure 26-29);

1. 20(S)-dammar-24-ene-3 β , 12 β , 19, 20 tetrol : the ion characters; 199, 321, 411, 501, 584 m/z.
2. 20(S)-dammar-24-ene-3 β , 12 β , 20 triol : the ion characters; 199, 323, 413, 503 m/z.
3. 20(S)-dammar-24-ene-3 β , 6 α , 12 β , 20 tetrol : the ion characters; 199, 321, 411, 501 m/z.
4. Ginsenoside Rb1: 199, 323, 413, 503 m/z.

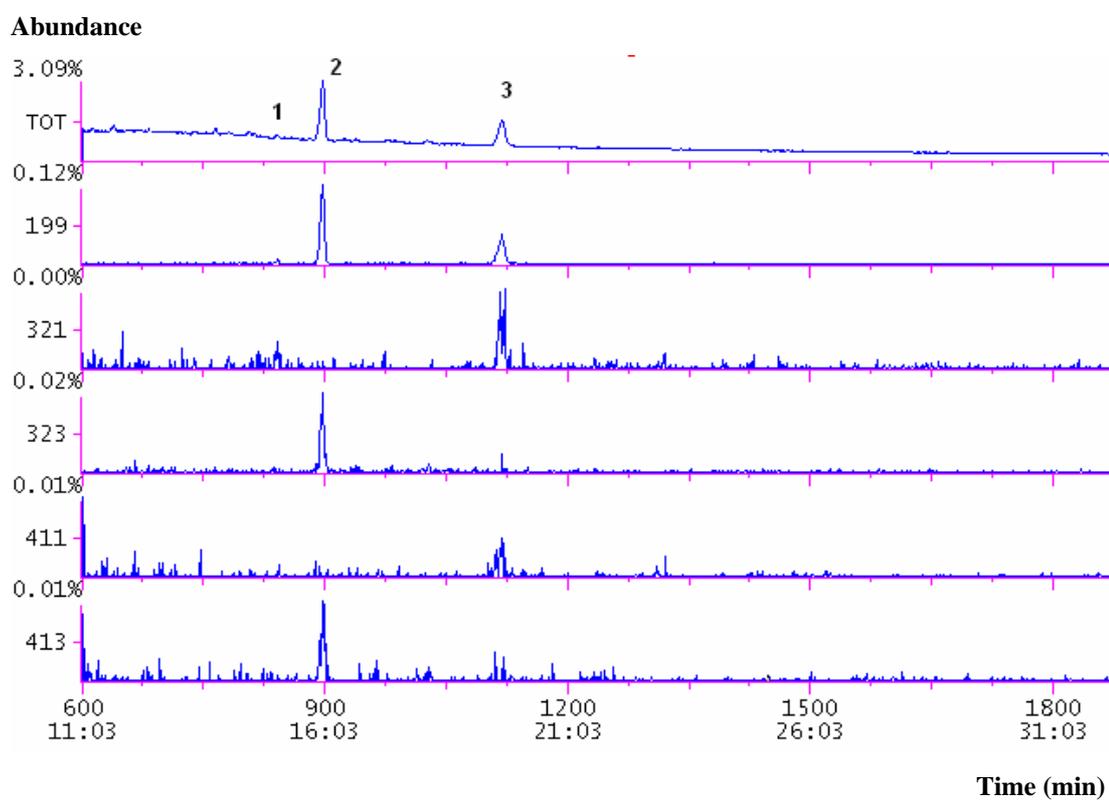


Figure 22 GC-MS ion chromatogram of Jiaogulan water extract.

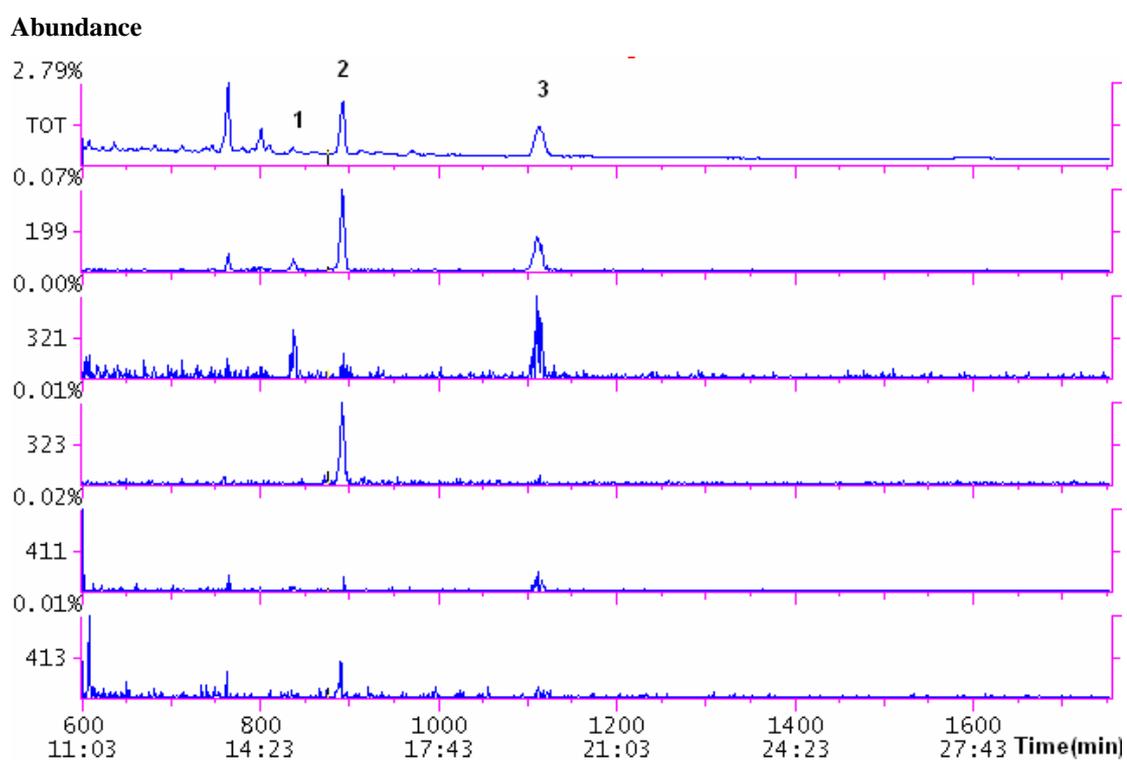


Figure 23 GC-MS ion chromatogram of Jiaogulan methanol extract.

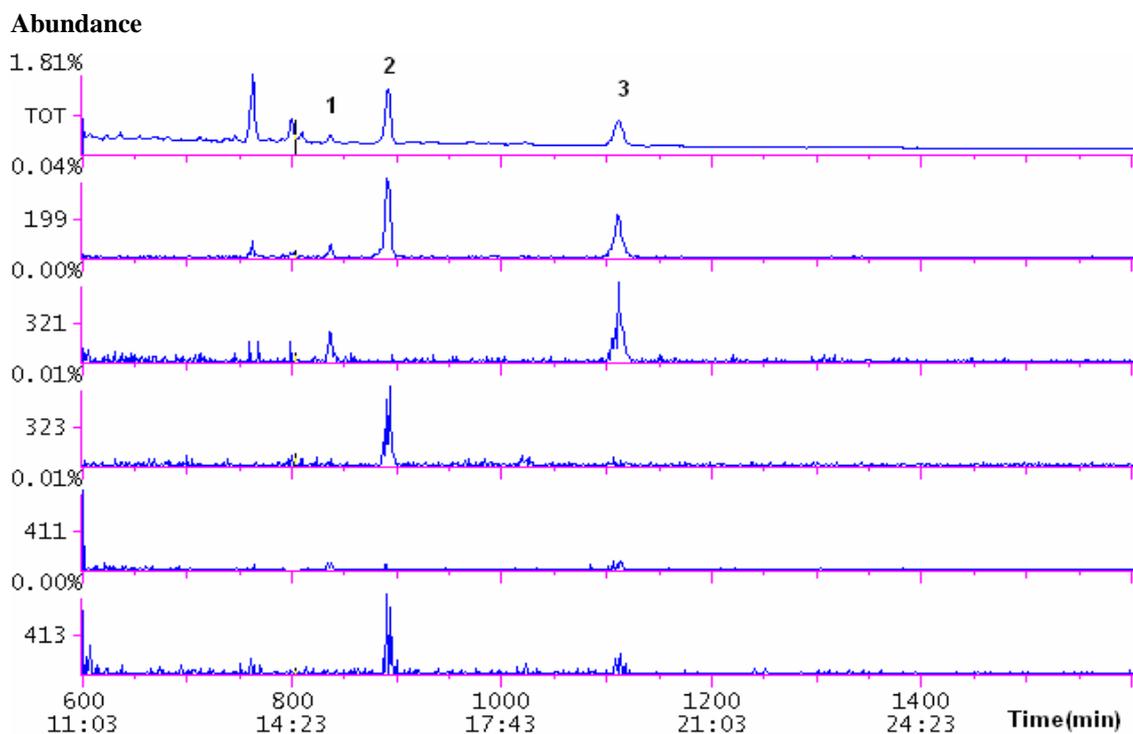


Figure 24 GC-MS ion chromatogram of Jiaogulan ethanol extract.

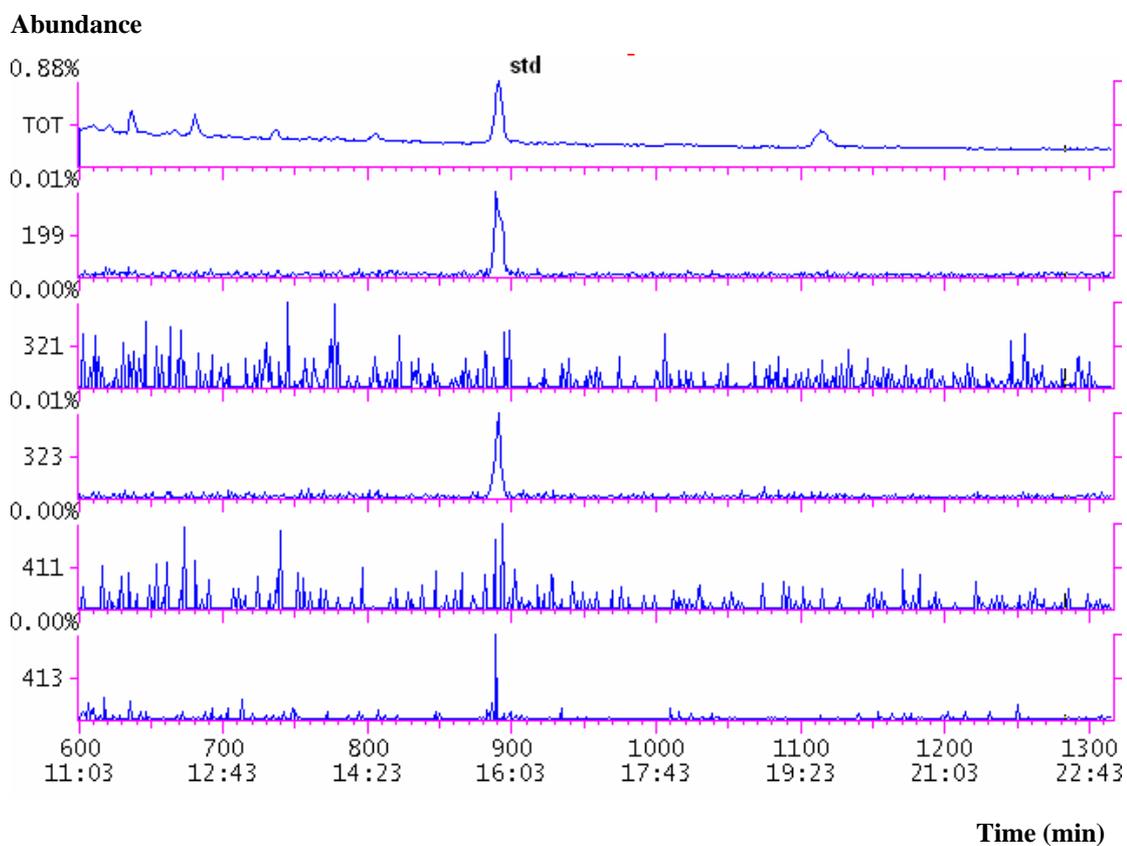


Figure 25 GC-MS ion chromatogram of standard ginsenoside Rb1.

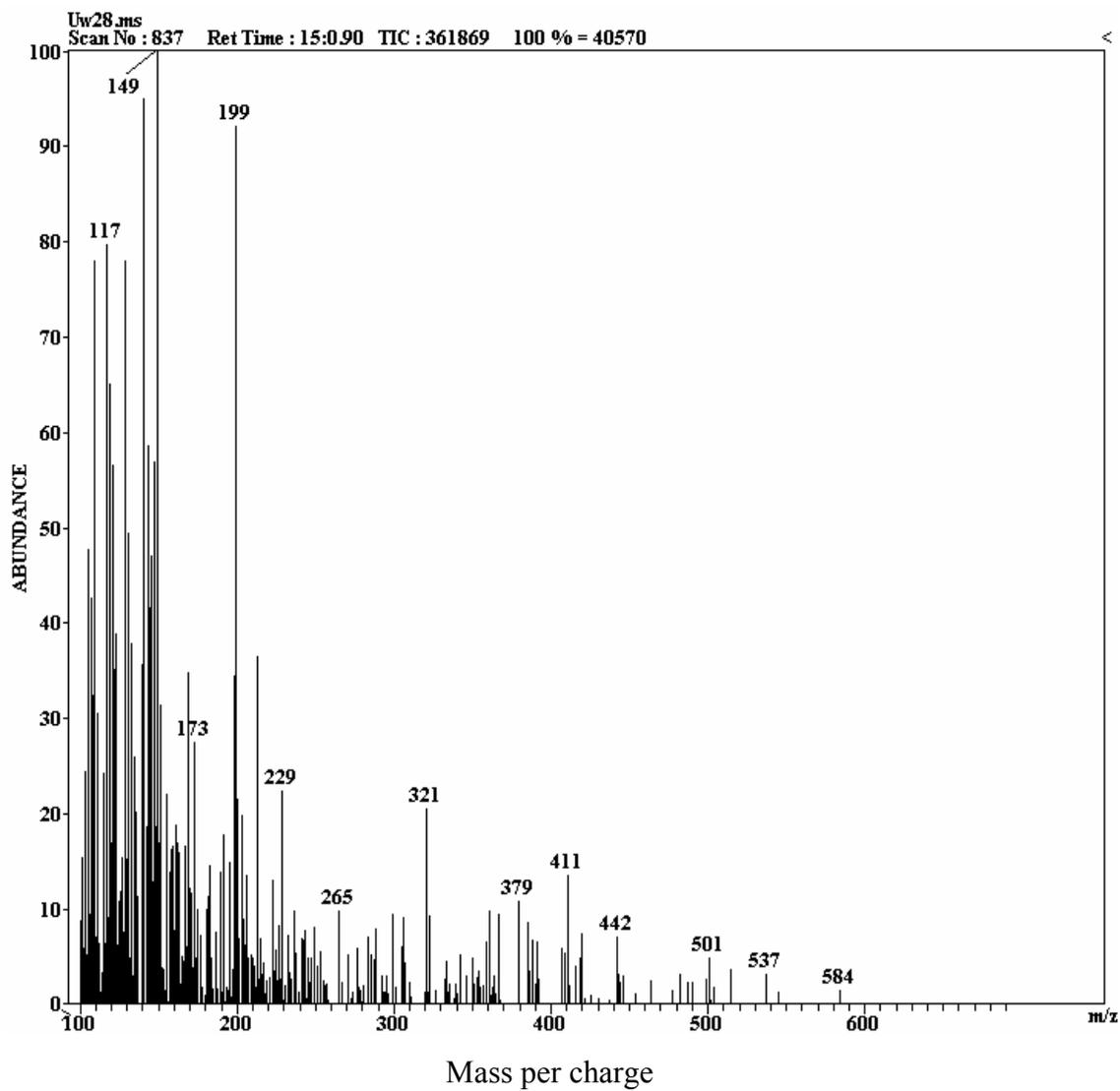


Figure 26 Mass spectrum of 20(S)-dammar-24-ene-3 β , 12 β , 19, 20 tetrol (Peak 1).

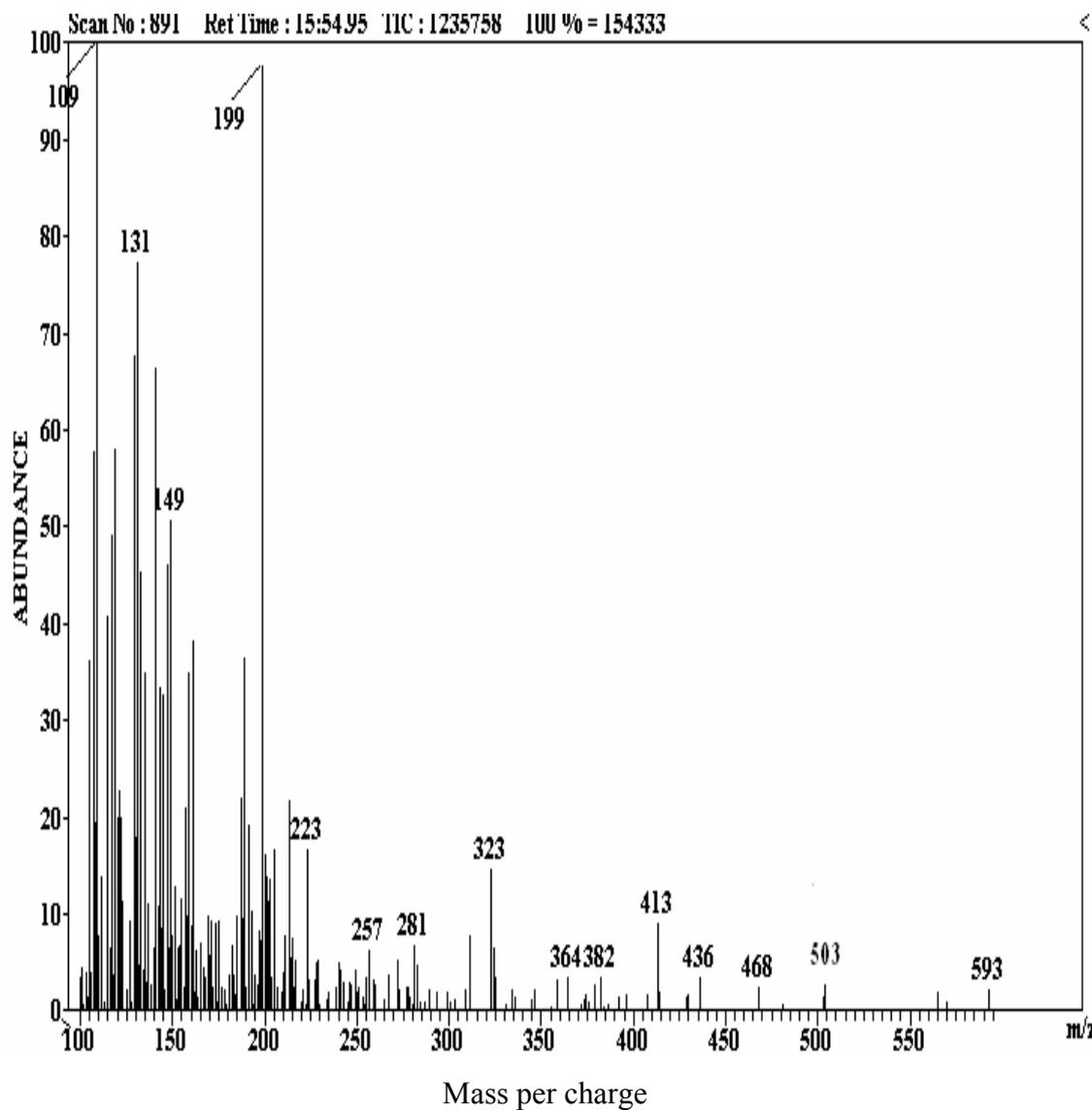


Figure 27 Mass spectrum of 20(S)-dammar-24-ene-3 β , 12 β , 20 triol (Peak 2).

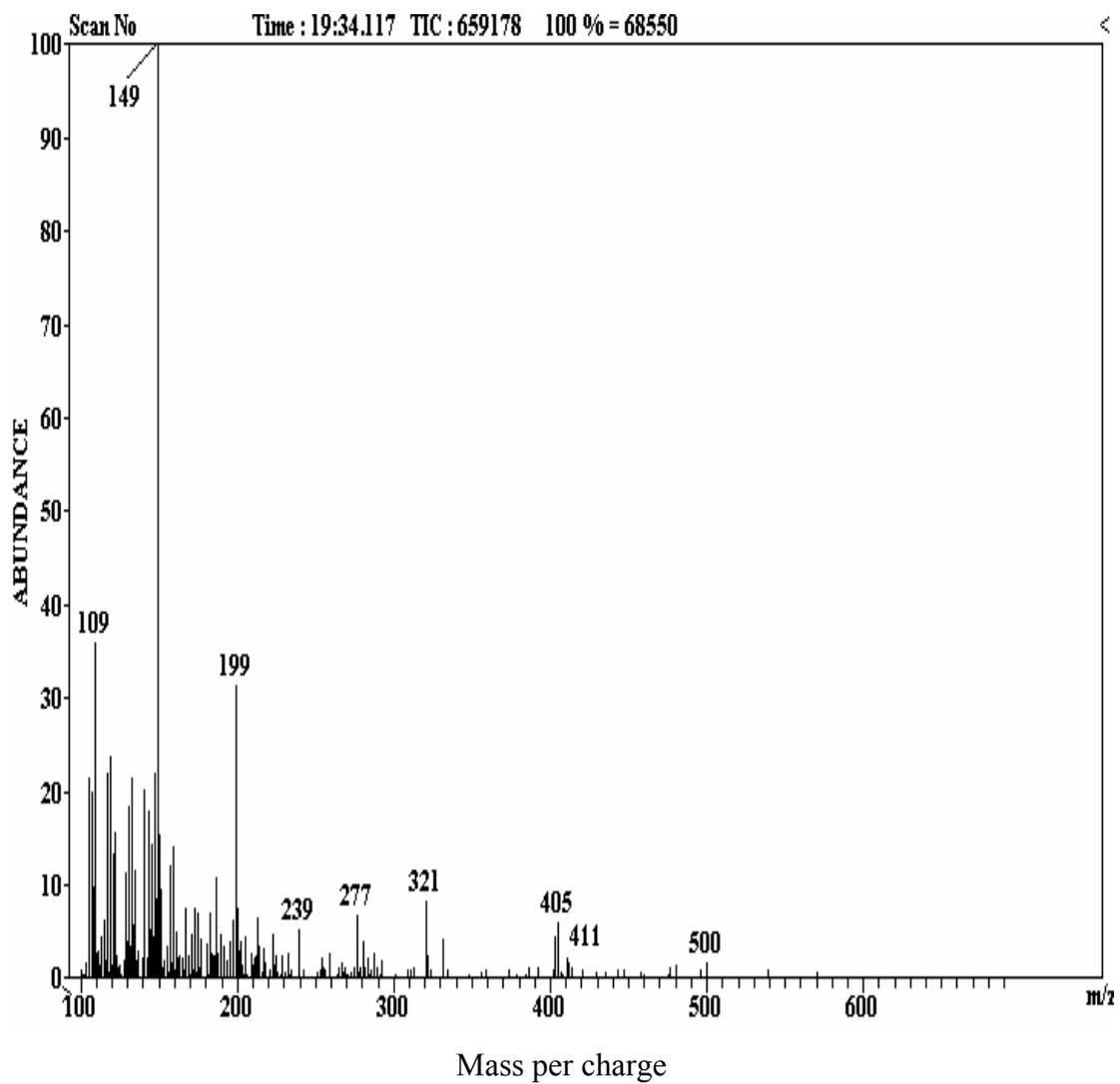


Figure 28 Mass spectrum of 20(S)-dammar-24-ene-3 β , 6 α , 12 β , 20 tetrol (Peak3).

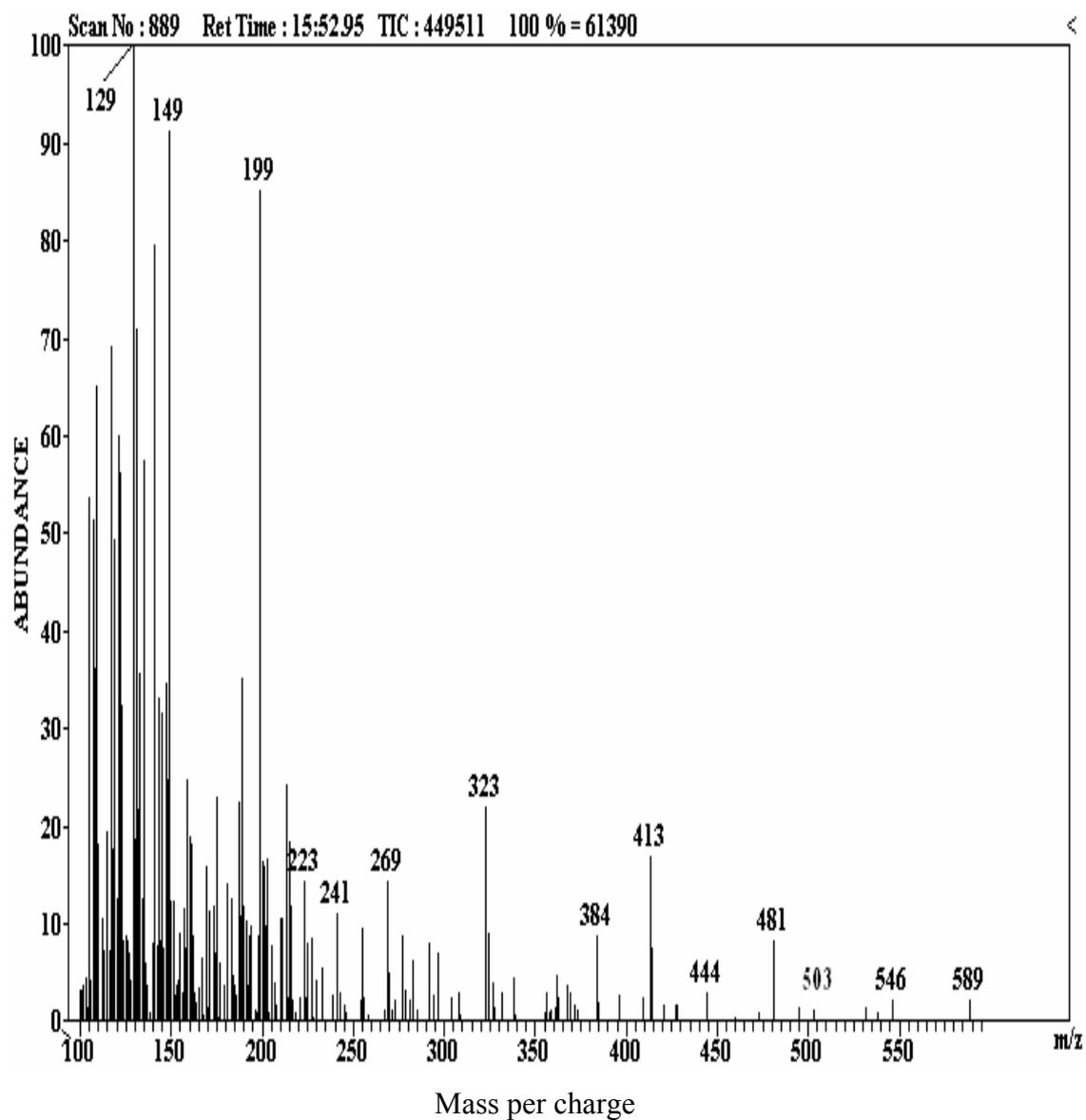


Figure 29 Mass spectrum of standard ginsenoside Rb1.

Table 12 presents molecular weight and the retention time of the aglycones obtained from Jiaogulan extracts and the standard Rb₁. The chromatograms show that the peak corresponding to the standard ginsenoside Rb₁ has the same retention time as the second peak found in the Jiaogulan extracts. Table 13 shows the characteristic ions of the trimethylsilylated aglycones from the mass spectral data. The structures of three aglycone saponins are shown in Figure 30. The first aglycone, 20(S)-dammar-24-ene-3 β , 12 β , 19, 20 tetrol, is not as pronounced in the water extract as in the methanol and ethanol Jiaogulan extract which consists of CH₂OH in the structure, so this compound has less polarity than the others. Cui *et al.* (1998) identified these aglycones from gypenosides LXII, LXIV, LXV, LXVI, LXXII, and LXXVI standards. The second dammar aglycone, 20(S)-dammar-24-ene-3 β , 12 β , 20 triol, is the most intense peak in all Jiaogulan extracts has the same retention time as the important compound from ginsenoside Rb₁ (Cui *et al.*, 1998). The third aglycone, 20(S)-dammar-24-ene-3 β , 6 α , 12 β , 20 tetrol, is supported by Cui *et.al.* (1998) who confirmed that it was a ginsenoside Rg₁. Previously, Cui *et al.* (1993) analyzed ginsenoside Rb₁ by chromatography and mass spectrometry which could produce two sapogenins, 20S-protopanaxadiol and 20S-protopanaxatriol, that had the same characteristics ion as our results. Cui *et al.* (1999) identified the major sapogenins from *Gynostemma pentaphyllum* compared to *Panax* species. This result supported our study that 20(S)-dammar-24-ene-3 β , 12 β , 19, 20 tetrol was found only in *G. pentaphyllum*, whereas 20(S)-dammar-24-ene-3 β , 12 β , 20 triol was found both in *G. pentaphyllum* and *Panax* species. While the 20(S)-dammar-24-ene-3 β , 6 α , 12 β , 20 tetrol was found only in *P. ginseng*, *P. quinquefolium* and *P. notoginseng* (Cui *et al*, 1999). Shen (2000) reported that ginsenoside Rb₁, one of the main 20(S)-protopanaxadiol group saponin, showed effective anti-inflammatory action, obvious vaso-dilating effect, and a tranquilizing effect on the central nervous system. Moreover, ginsenoside Rb₁ protected the brain from ischemic and reperfusion injuries (Zhang and Liu, 1996). 20 (S)-protopanaxatriol group, represented by ginsenoside Rg₁, possessed the properties of exciting the central nervous system, anti-fatigue and hemolysis (Shen, 2000).

In conclusion, this study shows that two major saponins in Jiaogulan water extract were the same compounds as in *Panax spp.* Two main saponins in Jiaogulan water extract were 20(S)-dammar-24-ene-3 β , 12 β , 20 triol and 20(S)-dammar-24-ene-3 β , 6 α , 12 β , 20 tetrol which are the same compound from ginsenoside Rb1 and Rg1 from ginseng (*Panax spp.*). In addition to these two compounds, one more compound was identified from the methanol and ethanol extract; which is 20(S)-dammar-24-ene-3 β , 12 β , 19, 20 tetrol.

Table 12 Aglycones obtained from Jiaogulan extracts by alkaline cleavage

Aglycones	MW ¹	t _R ² (min)	Base peak (m/z) ³
1. 20(S)-dammar-24-ene-3 β , 12 β , 19, 20 tetrol	476	15.01	199
2. 20(S)-dammar-24-ene-3 β , 12 β , 20 triol	460	15.55	199
3. 20(S)-dammar-24-ene-3 β , 6 α , 12 β , 20 tetrol	476	19.34	199
4. Standard Ginsenoside Rb1	460	15.53	199

¹ Molecular weight of aglycones.

² t_R = retention time.

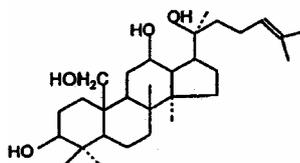
³ Base peak above 150 m/z.

Table 13 Characteristic ion of trimethylsilylated aglycones obtained from Jiaogulan extracts

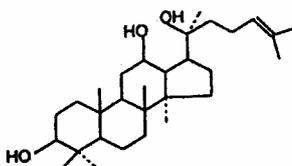
Aglycone derivative	MW ¹	Characteristic ion (m/z) ²
TMS-1 (20(S)-dammar-24-ene-3 β , 12 β , 19, 20 tetrol)	764	199, 321, 411, 501, 584
TMS-2 (20(S)-dammar-24-ene-3 β , 12 β , 20 triol)	676	199, 323, 413, 503, 593
TMS-3 (20(S)-dammar-24-ene-3 β , 6 α , 12 β , 20 tetrol)	764	199, 321, 411, 501
TMS-4 (Ginsenoside Rb1)	676	199, 323, 413, 503

¹ Molecular weight of trimethylsilylated derivatives of aglycones.

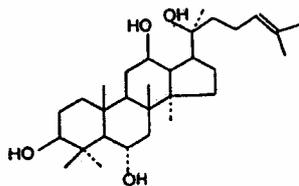
² mass-to-charge ratio.



(1) 20(S)-dammar-24-ene-3 β , 12 β , 19, 20 tetrol



(2) 20(S)-dammar-24-ene-3 β , 12 β , 20 triol



(3) 20(S)-dammar-24-ene-3 β , 6 α , 12 β , 20 tetrol

Figure 30 Structures of aglycones obtained some dammarane saponins isolated from Jiaogulan extracts

Source: Cui *et al.* (1999)

3.2 Toxicity Testing of Jiaogulan

The acute toxicity test was investigated in 10 mice (*Mus musculus* ICR strain). After consuming two doses (16.0 g/kg body weight), the mice showed no abnormality. The mice were observed for two weeks but there was no sign of toxicity and no severity. At the end of the 14-day-observation period (WHO, 2000), all animals survived and necropsy revealed no abnormality of the visceral organs. Therefore, the LD₅₀ of Jiaogulan extract are > 32.0 g/kg. The yield of extraction was at 39.82%. Hence, the Jiaogulan tea sample of more than 80.36 g made half of the mice died (50%). Normally, the limit dose is at least 2000 mg/kg (Interagency research animal committee, 1993; OECD, 2001). However, several countries have a requirement for information on toxicity at dose levels in the range 2000 to 5000 mg/kg for substances with LD₅₀ value in excess of 2000 mg/kg (OECD, 2001). From this result, the Jiaogulan sample is more than the standard, therefore, conclusion can be made that no indication for acute toxicity of Jiaogulan.

The chronic toxicity of *Gynostemma pentaphyllum* was studied by the Medicinal Plant Research Institute, Department of Medical Science, Ministry of Public Health, Thailand (Attawish *et al.*, 2004). Wistar rats who had coronically toxic blood for a period of six months. The water extract of *G. pentaphyllum* was fed at dose of 6, 30, 150 and 750 mg kg⁻¹ day⁻¹. The results showed that the hematological changes and the blood chemistry changes were still within the normal range, and there was not significant dose-related histopathological changes of the internal organs. Therefore, it is concluded that the extract of *G. pentaphyllum* at the given dosed did not produce any significant toxic effect in rats during a 6-month period.

In conclusion, Jiaogulan was nontoxic neither acutely nor chronically toxic. Thus it was safe to consume this plant as a food product.

3.3 Effect of Jiaogulan Water Extract on Serum Lipid in Animal Model

Body weight of experimented rats is presented in Figure 31. The mean body weights after each diet treatment were significantly different ($p \leq 0.05$); basal diet (232.37 ± 7.48 g), high cholesterol diet (236.95 ± 5.17 g), high cholesterol with Jiaogulan diet (245.32 ± 7.97 g). Food consumption of rats was significantly different ($p \leq 0.05$) from one diet treatment to the next. Rats consumed less of the cholesterol diet (43.41 ± 7.43 g/kg/day) compared to the basal diet (51.57 ± 12.42 g/kg/day) but non significantly different from the Jiaogulan diet (44.16 ± 4.12 g/kg/day).

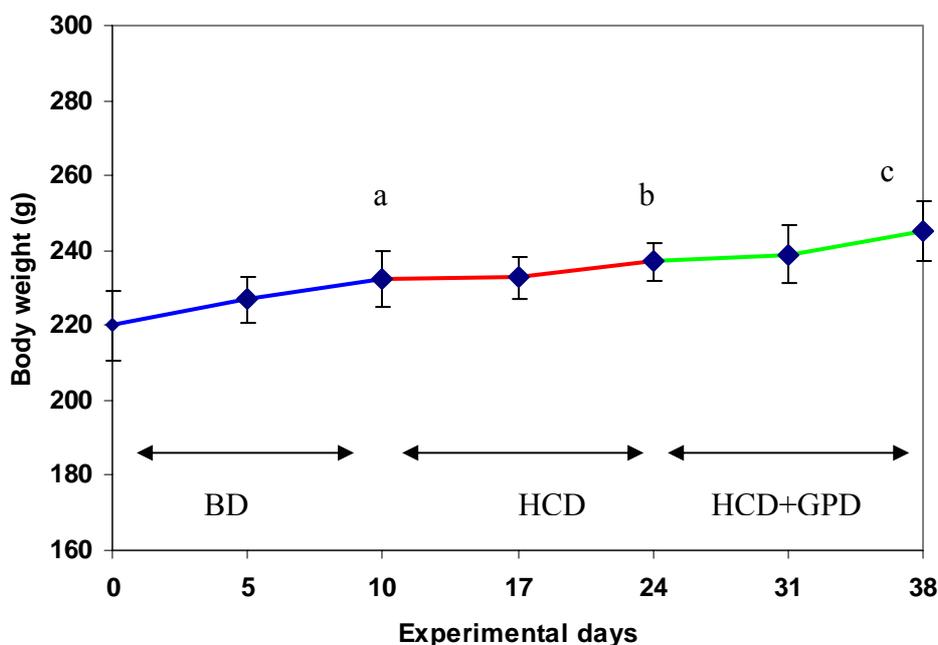


Figure 31 Average body weight of ten rats receiving the basal diet (0-10 days), the high cholesterol diet (11-24 days) and the Jiaogulan diet (25-38 days) (n=10).

Note: The different letters are significantly different at $p \leq 0.05$.

The diets: BD: basal diet, HCD: high cholesterol diet (1% cholesterol, 0.5% cholic acid), HCD+GPD: high cholesterol with GP diet (1%cholesterol, 3% Jiaogulan water extract).

In this experiment, the rats were fed the basal diet to adjust the base line of serum lipid before following the high cholesterol diet for 2 weeks, so the serum lipid profile increased. Then, the rats were fed a high cholesterol diet with a 3% Jiaogulan extract. The serum lipid profiles: total cholesterol (Table 14), triacylglycerol (Table 15), LDL cholesterol (Table 16) and HDL cholesterol (Table 17) were presented in mean and standard deviations. Table 18 presents the comparisons between the effects of diets by pair student's *t*-test. Total cholesterol was increased after feeding HCD and GPD but after 14 day of GPD the TC was non significantly different ($p > 0.05$) from the one started with HCD. This means that consuming Jiaogulan for longer period could stop the increase of total cholesterol levels. On the other hand, the triacylglycerols were not significantly different ($p > 0.05$) when the rats were fed all diets, but the final triacylglycerol level in the rats, after 14 days of GPD, was significantly ($p \leq 0.05$) lower than the triacylglycerol level in the rats after being fed the BD diet. Whereas HDL cholesterol was significantly ($p \leq 0.05$) decreased after feeding the rats HCD and GPD. In contrast, LDL cholesterol continued to increase but after the second week of GPD the increasing was stopped and trended to go down. Suppression was shown later in week 2 of the treatment indicating that the effect needed quite some time to occur. Therefore, it is possible that this is not the effect at the absorption step but rather the *de novo* metabolic effect. Furthermore, the serum lipid results from Jiaogulan extract were contrary to the effect of estrogen hormone in menopausal women that increased synthesis of triglycerides, increased in HDL and reduced in LDL (Greenspan and Strawler, 1997). Therefore, the Jiaogulan extract could reduce triacylglycerol but decrease HDL that produced the same effect as antiestrogen, such as tamoxifen, a partial estrogen agonist (Greenspan and Strawler, 1997; Clarke *et al.*, 2003). Currently, tamoxifen is the only drug approved for use in breast cancer chemoprevention and it remains the treatment of choice for most women with hormone receptor positive, invasive breast carcinoma (Clarke *et al.*, 2003).

Table 14 Total cholesterol level of the rats after various diet treatments

Rats	Total cholesterol level (mg/dl)			
	BD	HCD	GPD 7	GPD 14
R1	86.36	90.03	178.71	131.07
R2	88.48	116.86	NA	NA
R3	70.01	118.23	159.62	89.73
R4	116.18	158.75	NA	102.61
R5	99.92	116.13	202.23	232.39
R6	86.27	111.97	103.77	120.44
R7	77.77	109.46	134.08	135.93
R8	96.64	92.82	156.77	122.73
R9	99.25	77.22	165.61	176.40
R10	95.51	84.69	170.08	NA
R11	NA	131.95	143.84	145.49
R12	101.18	103.17	182.05	161.56
R13	NA	102.20	199.49	148.91
Mean±S.D.	92.51±12.56	108.72±21.43	163.30±28.81	142.48±39.25

Note: - The diets: BD: basal diet, HCD: high cholesterol diet (1% cholesterol, 0.5% cholic acid), GPD 7: high cholesterol with GP diet (1% cholesterol, 3% Jiaogulan water extract) for 7 days, GPD 14: high cholesterol with GP diet for 14 days).

NA = not available.

Table 15 Triacylglycerol level of the rats after feeding various diet treatments

Rats	Total triacylglycerol level (mg/dl)			
	BD	HCD	GPD 7	GPD 14
R1	41.04	37.34	32.13	25.12
R2	86.23	38.16	NA	29.82
R3	47.67	89.12	84.47	24.51
R4	53.31	34.05	NA	32.31
R5	70.15	NA	46.85	59.13
R6	77.75	27.50	25.29	35.69
R7	37.46	51.14	51.55	34.12
R8	48.57	49.87	59.6	52.42
R9	42.28	65.94	33.96	NA
R10	51.31	38.23	53.86	NA
R11	NA	94.64	137.81	51.12
R12	71.35	95.02	96.91	52.62
R13	NA	46.14	42.15	55.93
Mean±S.D.	57.01±16.50	56.52±23.77	52.68±22.81	41.23±12.58

Note: - The diets: BD: basal diet, HCD: high cholesterol diet (1% cholesterol, 0.5% cholic acid), GPD 7: high cholesterol with GP diet (1% cholesterol, 3% Jiaogulan water extract) for 7 days, GPD 14: high cholesterol with GP diet for 14 days).

NA = not available.

Table 16 LDL cholesterol level of the rats after feeding various diet treatments

Rats	LDL cholesterol level (mg/dl)			
	BD	HCD	GPD 7	GPD 14
R1	14.66	46.93	155.08	110.75
R2	10.03	89.97	NA	NA
R3	19.51	72.21	120.75	66.35
R4	21.54	NA	NA	81.46
R5	18.51	59.74	177.45	NA
R6	NA	88.14	83.25	104.56
R7	22.74	75.97	104.99	111.00
R8	25.35	52.77	124.68	95.90
R9	31.33	41.90	136.88	NA
R10	27.43	65.76	141.66	NA
R11	NA	88.37	85.83	120.51
R12	NA	61.31	141.36	138.92
R13	NA	70.39	169.89	123.90
Mean±S.D.	21.23±6.51	70.60±17.50	131.08±31.06	110.33±25.13

Note: - The diets: BD: basal diet, HCD: high cholesterol diet (1% cholesterol, 0.5% cholic acid), GPD 7: high cholesterol with GP diet (1% cholesterol, 3% Jiaogulan water extract) for 7 days, GPD 14: high cholesterol with GP diet for 14 days).

NA = not available.

Table 17 HDL cholesterol level of the rats after feeding various diet treatments

Rats	HDL cholesterol level (mg/dl)			
	BD	HCD	GPD 7	GPD 14
R1	63.49	35.63	17.20	15.29
R2	61.20	19.26	NA	15.41
R3	40.97	28.19	21.98	18.48
R4	83.98	NA	NA	14.69
R5	67.38	22.61	15.41	8.17
R6	NA	18.33	15.46	8.75
R7	47.54	23.26	18.78	18.11
R8	61.58	30.08	20.17	16.35
R9	59.46	22.13	21.94	11.09
R10	57.81	11.28	17.64	NA
R11	NA	24.65	30.45	14.76
R12	NA	22.85	21.30	12.11
R13	NA	22.58	21.17	13.82
Mean±S.D.	60.38±12.08	23.40±6.12	20.14±4.20	14.21±3.35

Note: - The diets: BD: basal diet, HCD: high cholesterol diet (1% cholesterol, 0.5% cholic acid), GPD 7: high cholesterol with GP diet (1% cholesterol, 3% Jiaogulan water extract) for 7 days, GPD 14: high cholesterol with GP diet for 14 days).

NA = not available.

Table 18 The mean comparisons of serum lipid after feeding various diets by Pair Student's *t*-test¹.

	Means (<i>t</i> -value)				
	BD-HCD	HCD-GPD7	GPD7-GPD14	HCD-GPD14	BD-GPD14
Total cholesterol	92.51-108.72 (-2.889 [*])	108.72-163.3 (-4.879 [*])	163.30-142.48 (1.695 ^{ns})	108.72-142.48 (-2.139 ^{ns})	92.51-142.48 (-3.208 [*])
Triacylglycerol	57.01-56.52 (0.589 ^{ns})	56.52-52.68 (-1.113 ^{ns})	52.68-41.23 (1.972 ^{ns})	56.52-41.23 (1.774 ^{ns})	57.01-41.23 (2.863 [*])
LDL cholesterol	21.23-70.60 (-5.743 [*])	70.60-131.08 (-5.086 [*])	131.08-110.33 (0.610 ^{ns})	70.60-110.33 (-4.358 [*])	21.23-110.33 (-5.042 [*])
HDL cholesterol	60.38-23.40 (8.178 [*])	23.40-20.14 (1.728 ^{ns})	20.14-14.21 (4.247 [*])	23.40-14.21 (4.894 [*])	60.38-14.21 (9.658 [*])

¹ Data present as means and *t*-value. The paired student's *t*-test was used to compare with their respective initial treatment by significantly different between two mean values at $p \leq 0.05$.

$T_{table} = 2.228$ at d.f.=10, $\alpha=0.05$.

^{ns} Non significant difference ($p > 0.05$).

The diets: BD: basal diet, HCD: high cholesterol diet (1% cholesterol, 0.5% cholic acid), GPD 7: high cholesterol with GP diet (1%cholesterol, 3% Jiaogulan water extract) for 7 days, GPD 14: high cholesterol with GP diet for 14 days).

Crude saponin isolated from *G. pentaphyllum* has been shown to reduce the serum level of triglycerides, lipid peroxide, total cholesterol, phospholipids, and glutamic pyruvic transaminase in animal studies (Kimura, 1983). La Cour *et al.* (1995) designed to evaluate the ability of a decoction of 3 species; *Crataegus cuneata*, *Nelumbo nucifera* and *G. pentaphyllum*; lower in cholesterol and triglycerides in a short-term experiment with rats and quails. The animals were fed high lipid diets and the herbal decoction for one week. The decoction of the plants was prepared from dried material by extracting with water, boiling for one hour, and storing under refrigeration. In rats an average of 20 replicates showed that the effect of *G. pentaphyllum* was not linear, but rather showed an optimum dose of 2 g/kg per day, for both cholesterol and triglycerides. Qi *et al.* (1996) investigated the influence of gypenosides on serum lipoprotein and atherosclerosis in hyperlipidaemia animals. Results indicated that gypenosides can suppress the rise of serum cholesterol and triglycerides in hyperlipidaemia mice and lower the content of cholesterol triglycerides and LDL in hyperlipidaemia quails. The hypocholesterlaemic activity of a different fraction from *Terminalia arjuna* were tested on rats fed an atherogenic diet. The result showed that the aqueous fractions, which consisted of saponin, flavonoids and phenolic compound, inhibited the rise in serum cholesterol, but the triglyceride levels were not significantly different. The fecal excretion of total bile acids in rats fed with this fraction was increased. The possible mechanism of action of the cholesterol-reducing activity of this fraction may be due to the rapid excretion of bile acids (Shaila *et al.*, 2000).

Saponins formed complexes with neutral sterols as part of plant defence mechanisms against pathogenic fungi, and bile acids form complexes with saponins as part of a mammalian defense mechanism against toxic saponins present in vegetables. A two-fold relationship exists between neutral sterols, saponin and bile acid could contribute to a net hypocholesterlaemic effect (Yves sauvaire *et al.*, 1991). Circosta *et al.* (2005) showed that the cardiovascular activity of the aqueous extract of *G. pentaphyllum* Makino leaves was investigated in the anaesthetized guinea-pigs and had been compared with two of its isolated gypenosides (III, VIII) and with verapamil, a well-known Ca-antagonistic drug. The results showed that the

intravenous administration of decoction of GP (2.5, 5 and 10 mg/kg) produced a protective effect against pitressin-induced coronary spasm, arrhythmias and pressor response. Extraction also increased the dose of ouabain required to cause ventricular tachyarrhythmias and lethality.

Yamamoto *et al* (1983) separated ginsenoside saponin fraction 3 and 4 and studied the effect on tumor transplant rats. The result showed that the plasma cholesterol, triglyceride and non-esterified fatty acid levels were reduced with oral administration. In incorporation of ^{14}C -acetate into total lipids, free and esterified cholesterol, triglycerides and phospholipids in liver was enhanced by ginsenoside saponin fraction 3 in both normal and tumor bearing rats. They studied the plasma lipid-lowering action of ginseng saponins and mechanism of the action. The results showed that the elevation of plasma levels of cholesterol and triglyceride was reduced by the intramuscular injection ginsenoside saponin fraction 4 (saponin content, ca $\frac{1}{2}$). The elimination of intraperitoneally injection 4- ^{14}C -cholesterol from plasma was accelerated by fraction 4 administration. Fecal excretion ^{14}C bile acids and ^{14}C sterols after intraperitoneal injection of 4- ^{14}C -cholesterol was significantly increased by fraction 4 administration.

On the other hand, in some researches, Jiaogulan did not affect to serum lipid levels. Attawish *et al.* (2004) studies the chronic toxicity of GP in male and female Wistar rats. The rats were orally treated with water extract at doses of 6, 30, 150 and 750 mg/kg/day. The results showed no significant difference in triglyceride and cholesterol levels with all doses after 6 month. Sinsatenporn *et al.* (2001) studied efficacy of GP in 23 patients with hyperlipidemia by orally administering GP powder in capsule at the dosage of 5 grams three times a day for 3 months. The results showed that the lipid profile (cholesterol, triglyceride, high density lipoprotein and low density lipoprotein) were not significantly different at month 1, 2 and 3. Oxidative stress & anti-oxidant activity and anti-platelet activity in the serum samples taken from patients were not significantly different.

In the current study, the saponins in Jiaogulan were the same as ginsenosides. Ginsenosides are the most important active constituents identified in all species of ginseng, including *Panax Ginseng* C.A. Meyer, *Panax quinquefolius* L. and *Panax notoginseng* (Banthorpe, 1994). There are two major classes of ginsenosides, namely, the derivatives of protopanaxadiol (Rb1, Rb2, Rc and Rd) and protopanaxatriol (Rg1, Rg2, Re and Rf). Rb1 shows effective anti-inflammatory action, obvious vasodilating effect and a tranquilizing effect on the central nervous system (Shen, 2000). Rb1 protected the brain from ischemic and reperfusion injuries (Zhang and Liu, 1966). Cho *et al.* (2004) studied the estrogenic activity of ginsenoside Rb1 from *Panax ginseng* C.A. Mayer. The activity of ginsenoside Rb1 was characterized in a transient transfection system, using estrogen receptor isoforms and estrogen-responsive luciferase plasmids, in COS monkey kidney cells. The results indicated that the estrogen-like activity of ginsenoside Rb1 was independent of direct estrogen receptor association (Cho *et al.*, 2004). Another class, Rg1 possessed the properties of exciting the central nervous system, anti-fatigue and hemolysis (Shen, 2000). Chan *et al.* (2002) determine that ginsenoside Rg1 could act like an estrogen analog in stimulating human breast cancer cell growth as well as in the activation of estrogen response element luciferase activity in HeLa cell. Therefore, the gypenoside in Jiaogulan might have an effect like ginsenoside Rb1 and Rg1 in estrogen activity to prevent breast cancer cell growth.

In conclusion, this study shows that the major saponins in Jiaogulan water extract are the same compound as ginsenoside Rb1 and Rg1 from *Panax* species. From the rat study, Jiaogulan water extract could lower total cholesterol and LDL cholesterol level when consumed for two weeks. But Jiaogulan did not affect HDL cholesterol level in the two weeks. Moreover, the Jiaogulan extract could significantly reduce triacylglycerol level within two weeks.

4. Phase IV: Product development of herbal beverage from Jiaogulan

4.1 Product survey

The survey was conducted from November to December, 2003. The purpose was to survey herbal beverage products on the market in Thailand. Table 19 shows the herbal products which were divided into 4 categories; dried herb, herbal infusion, herbal instant powder and sterilized herbal beverage. The dried herb was the original product which was processed by drying method. The herbal infusion product was the dried herbs which was ground into the fine particle and packed in the tea bag. The herbal instant powder was the dried herbal extract mixed with sugar or other ingredients which could easily dissolve in hot water. Finally, the sterilized herbal beverage was a ready-to-drink product which was packed in the UHT box and tin can. Based on the herb used, there are many Thai herbs such as chrysanthemum or Chinese aster, roselle, safflower, stevia, lemon grass, pandanus and ginger. Green tea is one of the most popular products in the market now. The green tea market grew very fast, starting in 2002 with 230 million Baht in sales and increased to 3,400 million Baht in sales in 2004. Oishi had the largest share of the market (62%) (Manager weekly, 2005). There are many green tea products such as original green tea, instant green tea, ice green tea, milk green tea, soy milk green tea and green tea mixed fruit. Individually, Jiaogulan herb is also in the market place but it is in the initial stage in the form of tea leaf or tea bag infusion. There were few producers of Jiaogulan products. From this survey, the great market of green tea product may be shared by other herbal beverage such as the beverage from Jiaogulan herb. Therefore, it was possible that Jiaogulan beverage products could expand in the market using product development system.

Table 19 Herbal beverage products in the market place in Thailand during November to December, 2003

Product forms	Brand	Product type	Manufacturing	Packaging	Weight/ Volumn	Price (Baht)
Dried herb/ dried leaf	Fujian	Jasmine tea	Zhe Jiang tea Imp&Exp.Co., Ltd., China	Rectangular aluminum box	100 g.	99
	HANA	Mullberry green tea	Green tea Ltd., Part., Bangkok	Cylinder plastic box	120 g	50
	Jasmine Tea	Jasmine tea	Onoiki tea Ltd., Bangkok	Paper box	100 g	40
		High class Jasmine tea	Onoiki tea Ltd., Bangkok	Paper box	100	53
	Doi Kharm / Royal Project	Chinese green tea	Angkhang Royal Project Station, Chiangmai	Cylinder can	85 g	150
	Project	Chamomile flower	Angkhang Royal Project Station, Chiangmai	Polyethylene bag	20 g	35
		Green tea	Angkhang Royal Project Station, Chiangmai	Aluminium foil bag	50 g	75
		Jiaogulan tea	Angkhang Royal Project Station, Chiangmai	Aluminium foil bag	50 g	75

Table 19 (Continued)

Product forms	Brand	Product type	Manufacturing	Packaging	Weight/ Volumn	Price (Baht)
Dried herb/ dried leaf	Sun-Cha	Chinese tea	China Immax Ltd., Bangkok	Paper box	120 g	23
	Three hoses	Tea leaf	Three horses tea Co., Ltd., Bangkok	Paper box	100 g	36
Herbal powder/ Herbal infusion/ Sachet	Abhaibuhu bejhr	Leng Ju Chao (Murdania lorifomis)	Chaphya Abhaibuhubejhr hospital, Prachinburi	Tea bag in Aluminium foil bag	10 tea bag x 1.5 g	35
		Safflower herbal tea	Chaphya Abhaibuhubejhr hospital, Prachinburi	Tea bag in Aluminium foil bag	10 tea bag x 2.5 g	50
		Cat's whisker herbal tea	Chaphya Abhaibuhubejhr hospital, Prachinburi	Tea bag in Aluminium foil bag	10 tea bag x 2.5 g	35
		Candelabra Bush herbal tea (<i>Senna alata</i> Linn)	Chaphya Abhaibuhubejhr hospital, Prachinburi	Tea bag in Aluminium foil bag	10 tea bag x 1.5 g	35
		Cha Plu herbal tea (<i>Piper samentosum</i>)	Chaphya Abhaibuhubejhr hospital, Prachinburi	Tea bag in Aluminium foil bag	10 tea bag x 2 g	35

Table 19 (Continued)

Product forms	Brand	Product type	Manufacturing	Packaging	Weight/ Volumn	Price (Baht)
Herbal powder/ Herbal infusion/ Sachet	Doi Kham	Mint tea	Royal Project Foundation, Chiangmai	Tea bag in paper box	25 tea bags x 1.5 g	125
		Lemon Thyme tea	Royal Project Foundation, Chiangmai	Tea bag in paper box	25 tea bags x 1.5 g	125
		Lemon Balm	Royal Project Foundation, Chiangmai	Tea bag in plastic box	10 tea bags x 1.5 g	65
		Chamomile tea	Royal Project Foundation, Chiangmai	Tea bag in paper box	10 tea bags x 1.5 g	65
		Mulberry herbal tea	Royal Project Foundation, Chiangmai	Tea bag in paper bag	30 tea bags x 1.5 g	70
		Jiaogulan tea	Royal Project Foundation, Chiangmai	Tea bag in paper box	25 tea bags x 1.5 g	65
	Lipton Yellow Label Tea	Tea powder	Uni-liver Thai Holding Ltd., Bangkok	Tea bag in paper box	50 g (25 tea bagx2g.)	44

Table 19 (Continued)

Product forms	Brand	Product type	Manufacturing	Packaging	Weight/ Volumn	Price (Baht)
Herbal powder/ Herbal infusion/	Ranong tea	Japanese green tea	Green Tea Ltd., Part., Bangkok	Tea bag in paper box	30 tea bags	96
	Tra Fitne'	Herbal Infusion Chrysanthemun	Tra Fitne' Ltd., Bangkok	Tea bag in plastic bag	112 g (40 tea bags)	113
	Twining's of London	Barl grey tea	Twining & Company Ltd., London	Tea bag in paper box	50 g (25 tea bags)	125
		Darjeeling tea	Twining & Company Ltd., London	Tea bag in paper box	50 g (25 tea bags)	125
		Finest Ceylon	Twining & Company Ltd., London	Tea bag in paper box	50 g (25 tea bags)	125
Instant powder / granular	Ginger	Instant ginger drink	Helic Vasher Food Product Ltd., Bangkok	Aluminum bag in paper box	70 g (14 bags x 5 g)	74
	HOTTA	Instant ginger	New Concept Product Ltd., Bangkok	Sachet in paper bag	252 g (14 sachets)	79

Table 19 (Continued)

Product forms	Brand	Product type	Manufacturing	Packaging	Weight/ Volumn	Price (Baht)
Instant powder / granular	HOTTA	Ginger with Ginkgo extract	New Concept Product Ltd., Bangkok	Sachet in paper bag	150 g (10 sachets)	118
		Ginger with Ginseng	New Concept Product Ltd., Bangkok	Sachet in paper bag	150 g (10 sachets)	118
	Jitraphon	Basel Fruit Instant drink	Jitraphon herbal group, Chiangmai	Polyethylene bag	400 g	70
		Mulberry / Morus indical	Jitraphon herbal group, Chiangmai	Polyethylene bag	500 g	70
	Mae Chaeng	Instant Matoom beverage(Basel fruit)	Mae Chaeng Co., Ltd., Bangkok	Instant powder in plastic bottle	250 g	45
		Instant ginger	Mae Chaeng Co., Ltd., Bangkok	Instant powder in plastic bottle	500 g	
	O-HI-O	Safflower drink	Greater Polymanufacturing Ltd., Nakron Pathom	Sachet in paper bag	120 g (10 sachet)	55

Table 19 (Continued)

Product forms	Brand	Product type	Manufacturing	Packaging	Weight/ Volumn	Price (Baht)
Sterilized herbal beverage	CHABAA	Chrysanthemum drink	Malee Bangkok Co., Ltd., Nakorn Pathom	Tin can	240 ml	13
	Chitralada	Basel Fruit drink	Royal Chitralada Project, Bangkok	Tin can	250 ml	16
		Ginger drink	Royal Chitralada Project, Bangkok	Tin can	250 ml	16
		Chrysanthemum drink	Royal Chitralada Project, Bangkok	Tin can	250 ml	16
		Tamarind drink	Royal Chitralada Project, Bangkok	Tin can	250 ml	16
		Lemongrass drink	Royal Chitralada Project, Bangkok	Tin can	250 ml	16
	Leader Price	Rosella drink	Wongtan Ltd., Samusakorn	PET bottle	330 ml	6.75
		Tamarind drink	Wongtan Ltd., Samusakorn	PET bottle	330 ml	6.75
		Chrysanthemun drink	Wongtan Ltd., Samusakorn	PET bottle	330 ml	6.75

Table 19 (Continued)

Product forms	Brand	Product type	Manufacturing	Packaging	Weight/ Volumn	Price (Baht)
Sterilized herbal beverage	Lipton Ice Tea	Flavor tea drink	Sermsuk Ltd., Pathomtani	Tin can	325 ml	12.50
		Tea with lemon	Sermsuk Ltd., Pathomtani	Tin can	325 ml	12.50
		Tea with honey lemon	Sermsuk Ltd., Pathomtani	Tin can	325 ml	12.50
	Malee Tropical	Chrysanthemun drink	Malee Sampran Public Company, Ltd., Nakorn Pathom	Tin can	240 ml	13
		Grass Jelly drink	Malee Sampran Public Company, Ltd., Nakorn Pathom	Tin can	240 ml	13
	OISHI	Green tea original	OISHI Treading Ltd., Pathomtani	PET bottle	500 ml	19
		Green tea with honey and lemon	OISHI Treading Ltd., Pathomtani	UHT box	250 ml	9
		Sugar free	OISHI Treading Ltd., Pathomtani	UHT Box	1000 ml	32
	Pokka	Oolong tea	Pokka Corporation Ltd., Singapore	PET bottle	500 ml	20
Oolong tea and sugar free		Pokka Corporation Ltd Singapore	Tin can	300 ml	15	

Table 19 (Continued)

Product forms	Brand	Product type	Manufacturing	Packaging	Weight/ Volumn	Price (Baht)
Sterilized herbal beverage	Sen-Cha	Green tea	Ajinomoto Ltd., Authaya	PET bottle	500 ml	19
		Green tea with honey	Ajinomoto Ltd., Authaya	Tin can	250 ml	11
	Tipco	Ice green tea	Tipco F&B Co., Ltd., Prachuapkirikhan	UHT box	250 ml	9
		Green tea with honey and lemon	Tipco F&B Co., Ltd., Prachuapkirikhan	UHT	1000 ml	32
		Green tea with Chrysanthemum	Tipco F&B Co., Ltd., Prachuapkirikhan	UHT	1000 ml	32
	Unif	Green tea original	Unif-President Thailand, Ltd., Nakron Pathom	PET bottle	500 ml	15
		Green tea with lemon	Unif-President Thailand, Ltd., Nakron Pathom	PET bottle	1500 ml	53
		Green tea with Honey	Unif-President Thailand, Ltd., Nakron Pathom	UHT box	250 ml	9
				UHT box	1000 ml	35
				Tin can	250 ml	11

4.2 Focus Group Discussion

The focus group discussion was conducted by 3 groups in Chiangmai and 3 groups in Bangkok on December, 2003. The method and questions are followed by the moderator's guide (Appendix 2). A summary of the responses are provided in Table 20. The responses show the attitudes of consumers about herbal beverages, including; definition of herbal beverage in their opinion, the purposes of drinking and the opinion about their health benefits especially cholesterol aspect. Likewise, the qualitative data are about consumer behavior, such as the herbal beverage type which consumers like to drink, the frequency of drinking and the places to buy herbal products. The main responses are about product profile, packaging profile and the sensory properties of herbal beverage that they want. At the end, all participants tested the Jiaogulan tea sample (Doi Kham, The Royal Project Foundation, Chiangmai) and comment about the product, what they likes and disliked about this product, and also the product that they need. Chambers and Smith (1991) supported the view that qualitative consumer research method could be used to investigate a wide rang of issues and obtain detailed information about consumer attitudes, opinions, perceptions, behaviors, habits and practices. Qualitative research usually needs to precede quantitative testing to help establish criteria for data collection and to follow quantitative testing to aid the explanation of quantitative data. Focus groups conducted before quantitative research will help define the problem (Resurreccion, 1998). Finally, all data of this focus group were used to develop the questionnaire for the consumer survey (Appendix 3).

Table 20 Attitude and behavior of consumer in herbal beverage

Consumer attitude and behavior	Response	Questions and choice in consumer survey
Definition of herbal beverage	<ul style="list-style-type: none"> - the beverage made from a single herb that benefit to health - the beverage made from mixed herbs or fruits or vegetable that benefit to health - the beverage which is a medicine and benefit to health - the beverage that may be good to health or toxic, so there is a caution by safety issue to the consumer. 	<ul style="list-style-type: none"> - Definition of herbal beverages in your opinion - the beverage makes from a single herb - the beverage makes from mixed herbs - the beverage provides health benefit
Favorite herbal beverage	<ul style="list-style-type: none"> - the leaf tea: green tea, Japanese tea, Chinese tea, black tea, mulberry tea and Jiaogulan tea - the ready-to-drink tea: green tea, ice tea - the ginger drink - the other herbal beverage: chrysanthemum or Chinese aster drink, lemon grass drink, rosella drink, - the hot drink made from fresh or dried herb by boiling: safflower, ginkgo, ginseng, mulberry leaf, 	<ul style="list-style-type: none"> - What is your favorite herbal beverage? - green tea, Chinese tea, ginger drink, rosella drink, chrysanthemum drink, bale fruit drink, lemon grass drink, safflower drink

Table 20 (continued)

Consumer attitude and behavior	Response	Questions and choice in consumer survey
Frequency of drinking	<ul style="list-style-type: none"> - drinking everyday, every morning, 1-2 glass a day - drinking 1-3 times per week - on occasions such as drinking in the restaurant, drinking by gift from someone - Hardly drink 	<ul style="list-style-type: none"> - How often do you drink herbal beverages? - > 3 times a week - 1-3 times a week - < 1 time a week
The buying place	<ul style="list-style-type: none"> - department store - supermarkets - convenient store - fresh market by home making - the health shop, the vegetarian shop - restaurant - drugstore, Chinese drugstore 	<ul style="list-style-type: none"> - Where do you buy herbal beverages? - department store, supermarket, convenient store, fresh market, vegetarian shop, drug store, Chinese drug store

Table 20 (continued)

Consumer attitude and behavior	Response	Questions and choice in consumer survey
The purpose of herbal beverage drinking	<ul style="list-style-type: none"> - for health benefit, improve health, stronger health - for thirsty , refreshment - for defecation - reducing cholesterol - curing the disease - blood improvement - alternative drinking, escape from carbonate drink, water replacement - preferring the flavor and aroma of individual herb 	<ul style="list-style-type: none"> - What is your purpose for herbal beverage drinking? - health benefit - curing the disease - normal beverage, thirsty, flavor
Opinions about herbal beverages for reducing cholesterol	<ul style="list-style-type: none"> - believe that herb can reduce cholesterol like the advertising - believe that some herb can reduce cholesterol because they tested by themselves depend on the quantity or dose of drinking - depend on the way of drinking if they used fresh better than heated herb which may destroy the active ingredient 	Do you agree that some herbal beverages can reduce cholesterol?

Table 20 (continued)

Consumer attitude and behavior	Response	Questions and choice in consumer survey
Opinions about herbal beverages for reducing cholesterol	<ul style="list-style-type: none"> - depend on the way of drinking if they used fresh better than heated herb which may destroy the active ingredient -depend on the labeling that confirm the effect or the guarantee or passing from the experiment or scientific test - don't believe at all - unnecessary depend on other factors such as food, eating style and exercise, 	<p>Do you agree that some herbal beverage can reduce cholesterol?</p> <ul style="list-style-type: none"> - I believe in some herbal beverage can reduce cholesterol level - I think it depend on food intake - I think it depend on the exercise
The herbal beverage profile	<ul style="list-style-type: none"> - fresh quality - natural product in tea leaf or dried herb that can brew in hot water - powder in tea bag than can infuse in hot water - instant beverage - ready-to-drink beverage, cool beverage - pasteurized beverage 	<p>What herbal beverage product style do you like?</p> <ul style="list-style-type: none"> - nature product, dried herb, leaf tea - powder in tea bag - instant beverage, instant powder - ready-to-drink, sterilized beverage

Table 20 (continued)

Consumer attitude and behavior	Response	Questions and choice in consumer survey
The sensory quality of herbal beverages	<ul style="list-style-type: none"> - natural flavor from the original herb, don't mix with others or seasoning - original herb flavor mixed with Jasmine, Chinese aster, cabomine, honey, lime or mixed fruit - concentrated herb - sweetness; less, moderately or high in sweetness - slightly bitterness - slightly sourness 	<p>What about the sensory quality of herbal beverage?</p> <ul style="list-style-type: none"> - natural flavor - mixed with Jasmine, Chinese aster, honey, lime
Opinion about Jiaogulan beverages	<ul style="list-style-type: none"> - high in flavor and aroma - tea aroma and ginseng aroma - accepted flavor and aroma, - accepted bitter test - high in bitter taste, bitter in aftertaste - slightly sweetness, like licorice or stevia - optimum mix with bitterness and sweetness - astringency and astringent aftertaste - rejection because of bitterness, astringent, aftertaste 	<p>Sensory attributes of Jiaogulan tea.</p> <ul style="list-style-type: none"> - Jiaogulan flavor, aroma - Bitterness - Sweetness - Astringency

4.3 Consumer Survey

4.3.1 Demographic Data

The demographic profile of the respondents is shown in Table 21. There were 44.5% males and 55.5% females. The ages were divided into four groups (approximately 25% for each group) between 35-45, 46-55, 56-65 and older than 65 years old. Respondents were from four locations in Thailand; central Bangkok (n=99), northeastern (n=102), southern (n=108) and northern (n=107). The highest education indicated for most of them was bachelor degree (34.9%), followed by high school diploma (19.7%), primary school graduate (17.1%), an advance degree (14.2%) and a vocational degree (13.2%). The most of them had income higher than 20,000 Baht per month (35.65%).

Table 21 The demographic profile of the respondents¹ in consumer survey

(n = 416)

Respondent characteristic	Response (%)	Respondent characteristic	Response (%)
Gender		Education	
Female	55.5	Bachelor degree	34.9
Male	44.5	High school	19.7
Age (years)		Primary school	17.1
35-45	26.4	Advance degree	14.2
46-55	25.5	Vocational degree	13.2
56-65	24.3	Income (bath/month)	
> 65	23.8	< 5,000	8.2
Location		5,001-10,000	14.4
Southern	26.0	10,001 – 15,000	13.9
Northern	25.7	15,001 – 20,000	15.1
Northeastern	24.5	> 20,000	35.6
Central	23.8		

¹ Respondents involved in consumer survey in Thailand during January – February, 2004.

4.3.2 Consumer Behavior

Table 22 shows consumer behavior that their desires for drinking herbal beverages were to promote their health (49.0%), for use as a common beverage (42.1%) and to cure sickness (8.9%). According to the survey of Lappalainen *et al.* (1998), it was found that health is one of the frequently mentioned motivations behind food choices. Respondents drank herbal beverages 1-3 times/ week (38.5%), more than 3 times/ week (35.3%), and less than 1 time a week (26.2%). The favorite places respondents buy herbal beverage were department stores and supermarkets (34.7%), and convenience shops (24.5%) but some of them bought it from vegetarian shops, trade fairs and fresh markets. Results showed that these were popular places because they were comfortable places to buy these products in big cities. Table 23 shows the favorite herbal beverage by frequency of ranking (rank 1= the most liking and rank 3= the least liking) and score of liking. The most liked herbal beverage which consumer rank was green tea or Japanese tea (392 scores). The second favorite was a ginger drink (384 scores), the third was a Chinese tea drink (297 scores), and the fourth was chrysanthemum drink (295 scores). This result supported by Manager weekly (2005) was that the green tea market was a fast growing market which grossed 3,400 million Baht in 2004. Therefore, green tea is fashionable for Thai consumers now.

The attitudes of consumers are shown in Table 24. The respondents thought that herbal beverages should be made from mixed herbs (72%) rather than from one single herb (51.4%). They also thought that fruit (67.5%) and vegetable (73%) juice are types of herbal beverages, too. Most of the respondents believed that the herbal beverages should contain active ingredients which were advantage to our health (77%), but some of them wondered about side effects and toxicity of herbs (34.2%). In fact, the benefits and risks of herbal/specialty supplement use remain a controversial topic (Gunther *et al.*, 2004). Therefore, most consumers were concerned about safety (89.3%) and labeling (89.6%) of this product. More than half of the respondents believed that some herb could reduce cholesterol but that depended on the food intake and exercise, as well. Gunther *et al.* (2004) surveyed the use of herbal supplements such as ginkgo biloba and garlic pills and

explained that the health effects of using herbal supplements may be compromised if their use is mitigated by other factors such as diets or physical activity that affect disease risk. Finally, Thai respondents still believed in the ancient ideas about properties of herbs (69.2%). This finding compared to the study of Gordon *et al.* (2000) who surveyed Korean-American consumers about their dietary habits and health beliefs. Even though Koreans preferred Western medicine to traditional medicine, more than half of them used ginseng products and consumed hot ginger tea as a cold remedy.

In conclusion, the attitudes of Thai consumers were similar to the attitudes of other Asia group. That was still believed that herbs were beneficial to health, despite concerns over side effects and toxicity of herbs and despite reliance on modern medicine for other health concerns.

Table 22 The consumer behavior on herbal beverage of the respondents¹

(n = 416)

Behavior	Response (%)
Purpose of drinking	
Health	49.0
Common beverage	42.9
Curing the disease	9.1
Drinking frequency	
1 – 3 times/week (drink often)	38.5
> 3 times/week (always drink)	35.3
< 1 times/week (drink sometimes)	26.2
Drinking Time	
Morning	46.8
Afternoon	39.2
Noon	26.7
Night	25.5
Buying place	
Department store or supermarket	34.7
Convenience shop	24.5
Vegetarian shop	9.7
Trade fair	8.0
Home made	6.6
Fresh market	6.3
Other	10.2

¹ Respondents involved in consumer survey in Thailand during January – February, 2004.

Table 23 The favorite herbal beverage of respondents¹

Herbal beverage	Frequency of ranking ²			Score ³
	1	2	3	
Green tea /Japanese tea	89	46	33	392
Ginger drink	68	59	62	384
Chinese tea	54	34	25	297
Chrysanthemum drink	48	59	33	295
Lime drink	25	38	46	197
Lemon grass drink	23	38	33	178
Bourbok drink	22	38	36	178
Basel fruit drink	10	20	34	104
Ginseng drink	10	5	11	51
Safflower drink	8	9	8	50
Jabrain drink	7	6	19	43
Ganodenma drink	5	6	6	33
Kachaidum drink	3	5	11	30
Noni drink	5	3	5	26
Other	1	2	6	13

n=416

¹ Respondents involved in consumer survey in Thailand during January – February, 2004.

² Ranking: 1 = the most liking and 3 = the least liking.

³ Score = Sum of rank order x weight of score

Weight of score: 1 = 3 scores

2 = 2 scores

3 = 1 scores

Example: Green tea score = (89 x 3) + (46 x 2) + (33 x 1) = 392 scores

Table 24 Attitudes of the respondents¹ toward herbal beverage

(n = 416)

Statement	% of respondents		
	Agree	Neither agree Nor disagree	Disagree
1. Herbal beverages are beverages that consist of a single herb.	51.4	27.6	21.0
2. Herbal beverages are beverages that consist of mixed herbs.	72.0	22.0	6.0
3. Orange juice, lemon juice and fruit juice are herbal beverage.	67.5	16.7	15.8
4. Carrot juice, celery juice and vegetable juice are herbal beverages.	73.0	18.2	8.8
5. Herbal beverages provide health benefit or cure the diseases.	77.0	16.7	6.3
6. Herbal beverages provide only benefits. No bad effects.	53.4	34.2	12.4
7. Herbal beverages should be saved for drinking.	89.3	8.0	2.7
8. I read the label before drinking herbal beverages.	89.6	8.2	2.2
9. I believe that some herbs, such as green tea or safflower, can reduce cholesterol levels.	62.7	35.1	2.2
10. I think that lowering the cholesterol level depends on food intake.	84.5	11.2	4.3
11. I think that lowering the cholesterol level depends on exercise.	85.7	10.7	3.6
12. I believe in the ancient idea about the properties of herbs.	69.2	28.9	1.9

¹ Respondents involved in consumer survey in Thailand during January – February, 2004.

The factor analysis technique is used to reduce a large number of variables to a small set of new variables, called factors, which can be used to explain the variation in the data (Resurreccion, 1998). The average weighting score of factors is shown in Table 25. The important aspects that consumers concerned were about safety of herb, properties of herb, the label of herbal beverage and the side effect of herb. Twenty variables were divided into six factors that affected consumption behavior of herbal beverages by the factor analysis method (Table 26). The principle component analysis (PCA) could extract the factors from the correlation matrix (Federer, 1987; Jones, 1997) and varimax rotated into 6 components on the grounds of eigen values ($\lambda > 1$). It is then necessary to decide which eigen value are large (>1) that indicates the effective dimensionality of the data (Heslett, 2001). Together, the 6 factors explained 59.18% of the variance in the data. The new components were the sensory factor, belief factor, knowledge factor, herbal factor, marketing factor and consumption factor. For the sensory factor, consisted of the attributes color, odor, taste and sweetness had the highest positive loadings that could explain greater than other factors. The belief factor consisted of ideas about ancient beliefs, traditional medicine and modern medicine. The knowledge factor consisted of the consumer received information such as side effects, the natural products, packaging and labeling. The herbal factor consisted of the herbal type, property, safety and cleanliness of the herbal beverages. The marketing factor consisted of pricing, advertising and consumption convenience of the product. And the last factor was the pattern of consumption such as quantity and time of consumption. The relation models of each component are shown in Table 27. From this result, the Jiaogulan beverage concept could be developed in accordance with consumer ideas about sensory properties and health benefits of the herbal product.

Table 25 Factors that respondents weight for the important of the herbal beverage

(n=408)

Factor	7. The most important	6. Very important	5. Important	4. Neither important nor unimportant	3. Unimportant	2. Very unimportant	1. The most unimportant	Total	Average
Type of herb	114	115	143	29	4	1	2	2335	5.72
Properties of herb	141	125	123	18	1	0	0	2437	5.97
Safety of herb	245	98	55	6	2	1	1	2611	6.40
Side effect or bad effect of herb	157	96	102	32	15	3	3	2367	5.80
Color of herbal beverage	47	73	125	80	64	9	10	1932	4.74
Aroma of herbal beverage	58	93	161	52	35	8	1	2099	5.14
Taste of herbal beverage	68	119	142	40	34	3	2	2170	5.32
Sweetness of herbal beverage	35	51	118	91	86	16	11	1806	4.43
Natural flavor of herb beverage	144	95	105	38	21	3	2	2326	5.70
Quantity of herbal beverage drinking	50	88	170	57	35	7	1	2076	5.09
Time of herbal beverage drinking	55	100	146	57	43	6	1	2085	5.11

Table 25 (Continued)

Factor	7. The most important	6. Very important	5. Important	4. Neither important nor unimportant	3. Unimportant	2. Very unimportant	1. The most unimportant	Total	Average
Taste of herbal beverage	68	119	142	40	34	3	2	2170	5.32
Package of herbal beverage	90	108	135	50	17	7	1	2219	5.44
Label of herbal beverage	163	103	110	21	8	1	2	2421	5.93
Price of herbal beverage	78	76	162	48	33	7	4	2121	5.20
Advertising of herbal beverage	53	69	127	85	47	19	8	1947	4.77
Convenient of consumption	67	100	166	45	23	6	1	2161	5.30
Ancient belief	58	90	136	94	26	2	2	2086	5.11
The traditional doctor's suggestion	55	76	144	94	28	6	5	2038	4.99
The modern medicine doctor's suggestion	102	99	145	48	10	2	2	2261	5.54

Table 26 The factor loading of herbal beverage

Attributes	Factors	Components					
		1	2	3	4	5	6
Color	Sensory	0.71	0.11	0.19	-0.07	0.13	-0.01
Odor	Factor	0.77	0.08	0.24	0.14	-0.01	-0.08
Taste		0.75	-0.03	0.07	0.20	0.15	-0.13
Sweetness		0.66	0.17	0.31	0.07	0.12	0.36
Viscosity		0.59	0.11	0.09	-0.03	0.04	0.17
Ancient beliefs	Belief	0.12	0.80	0.32	0.08	0.06	0.14
Traditional medicine		0.12	0.84	0.15	0.08	0.05	0.08
Modern medicine		0.09	0.62	0.08	0.09	0.31	-0.12
Side effect or disadvantage	Information of Product	0.17	-0.26	0.43	0.36	0.26	-0.07
Natural product		0.15	0.19	0.64	0.05	-0.15	0.17
Packaging		0.13	0.17	0.67	0.02	0.03	0.12
Labeling		-0.01	0.13	0.54	0.21	0.36	-0.05
Herb type	Herbal type	0.10	0.17	-0.17	0.78	-0.05	0.17
Herbal property		0.10	0.16	0.08	0.82	0.01	0.10
Safety and clean		-0.01	-0.12	0.36	0.59	0.29	-0.10
Pricing	Marketing	0.13	0.00	0.06	0.02	0.77	0.11
Advertising		0.21	0.31	-0.09	0.03	0.70	0.09
Convenience		0.00	0.31	0.33	0.05	0.42	0.16
Volume	Consumption	0.001	-0.01	0.03	0.10	0.10	0.77
Drinking time		0.07	0.20	0.39	0.14	0.14	0.58
Eigenvalue (λ)		4.58	1.92	1.74	1.29	1.22	1.09
Variance explained (total 59.18%)		13.16	11.13	10.01	9.57	8.76	6.56

Extraction method : Principal Component Analysis (PCA)

Table 27 The relation model of components by factor analysis

Components	Models
Sensory factor	= 0.29 Color +0.32 Odor + 0.31 Taste +0.27 Sweetness
Belief factor	= 0.39 Ancient belief + 0.44 Traditional medicine + 0.30 Modern medicine
Information of product	= 0.21 Side-effect + 0.39 Natural product + 0.39 Packaging + 0.26 Labeling
Herbal type factor	= 0.48 Herbal type + 0.48 Herbal property + 0.29 Safety
Marketing factor	= 0.52 Pricing + 0.44 Advertising + 0.44 Convenience
Consumption factor	= 0.65 Volume + 0.43 Time

4.3.3 Sensory Acceptance Tests

The mean hedonic ratings and standard deviation of Jiaogulan beverage as rated by consumer (n=416) are presented in Table 28. Color, odor and taste were rated between *like slightly* to *like moderately*. And the overall acceptability was rated as *slightly like* that meant they accepted this product. Grosso and Resurrecion (2002) pointed out that a rating lower than 5 on a 9-point hedonic scale can be used to decide if a food is unacceptable to the consumer. Confirmed with the acceptance criteria, 80% respondents accepted the product and 20% rejected it.

The binary logistic regression was used to analyze the impact of hedonic attribute to the acceptance (Walker and Prinyawiwatkul, 2002). The results shown in Table 29 that taste and overall acceptability affected the acceptance of Jiaogulan beverage (P< 0.001). The odd ratio of taste and overall liking were 1.135 and 1.633. These mean that the probability acceptance was higher than the rejection. If the overall rate increases by 1 on the scale, the acceptance will be 1.6 times higher. The acceptance model was as follows:

$$Y = - 2.52 - 0.08 \text{ Color} + 0.13 \text{ Aroma} + 0.30 \text{ Flavor}^* + 0.49 \text{ Overall liking}^*$$

Where Y was the acceptability of Jiaogulan sample (0= reject and 1= accept)

Table 28 Mean hedonic ratings¹ of Jiaogulan tea as rated by consumers in different locations

Attributes	Locations				
	Bangkok (n=99)	Songkha (n=108)	Kongkean (n=102)	Chiangmai (n=107)	Average (n=416)
Color ^{ns}	6.04±1.89	6.32±1.81	6.18±1.97	5.93±2.10	6.12±1.95
Aroma ^{ns}	6.30±1.71	6.42±1.61	6.32±1.81	6.02±1.85	6.26±1.75
Flavor ^{ns}	6.16±1.95	6.48±1.84	6.43±1.65	6.31±1.88	6.32±1.83
Overall liking ^{ns}	6.39±1.78	6.61±1.61	6.26±1.88	6.26±1.86	6.38±1.79

¹ Ratings are based on a 9-point hedonic scale 1 = dislike extremely, 5 = neither like nor dislike and 9 = like extremely (Peryam and Pilgrim, 1957)

^{ns} non significant difference in the locations(P>0.05)

Table 29 Binary Logistic regression analysis of hedonic attribute rating effect to acceptance¹ of the respondents²

Attribute	Beta	Wald chi-square	Sig. (p<0.05)	Odd ratio ³
Color	-0.08	0.71	0.40	0.92
Aroma	0.13	1.17	0.28	1.14
Flavor	0.30	7.68 *	0.006	1.35
Overall liking	0.49	21.37 *	0.00	1.63
Constant	-2.52	18.39	0.00	0.08

n=416

¹ Dichotomous variables, 0= reject and 1 = accept.

² Respondents involved in the consumer survey in Thailand during January – February, 2004.

³ Odd ratio = P (accept) / P (reject)

The JAR scales are used in product development to adjust the intensities of specific attributes to levels that consumers say will be nearer the optimum (Bower and Boyd, 2003). For the Jiaogulan beverage sample, the cut-out point was set at a 50% response. Percent response of JAR scales were presented in Table 30 for all attributes; color, herbal odor, greenly odor, bitter, sweetness and astringent, were just right at 67.6%, 53.8%, 59.6%, 59.6%, 57.5% and 62.0%, respectively. That means this beverage was just right in color, odor and taste. Even though, the bitterness affected acceptance of food and beverages (Mattes, 1994) and Akella et al. (1997) showed sensory acceptance of Japanese green tea may be influenced by genetic sensitivity to the bitter taste of 6-N-Propylthiouracil. While, Stein et al. (2003) suggested that repeated exposure can enhance hedonic evaluation of a bittersweet beverage. So, the bitterness of Jiaogulan beverage could be accepted by Thai consumers.

Table 30 Response (%) in JAR scale for the attributes of Jiaogulan tea

Attributes	(n=416)				
	Much too little	Somewhat too little	Just right	Somewhat too much	Much too much
Color	3.4	12.0	67.6	13.2	3.8
Herbal odor	3.6	24.5	53.8	14.4	3.6
Greenly odor	1.7	5.0	59.6	21.9	11.6
Bitter	1.4	7.9	59.6	22.1	8.9
Sweetness	1.7	21.9	57.5	15.6	3.4
Astringent	1.7	5.3	62.0	22.6	8.4

4.3.4 Targeting Consumer of Jiaogulan Beverage

Table 31 shows the age and hedonic rating of attributes that there were not significantly different in color and odor. But, the liking score of taste and overall liking were significantly different ($p \leq 0.05$) between age groups. The liking score of 35-45 yr group was significantly lower than the other age group, so this group should not be selected as consumers of Jiaogulan beverages. Popper and Kroll (2003) reported about food preference and consumption among the elderly consumer; their sensory perception and their food preferences shifted with age. Supported by Murphy and Gilmore (1989), bitterness perception seemed to show the greatest decline with age and sweetness perception the least. Tepper et al. (1997) showed that older consumers, who expressed more concern about health, rated taste as less important for a variety of foods. Bower and Boyd (2003) studied the effect of health concern and consumption patterns on measures of sweetness by hedonic and just-about-right scales. Finally, Mattes (2002) described the chemical senses and nutrition in aging. Assumption 1. was about aging processes resulting in marked changes of chemosensory function and assumption 2. was about sensory changes associated with aging which translate into modifications of food choice and dietary behavior.

Table 32 presents the cross tabulations between age and their need for development of Jiaogulan beverage. The age groups, 56-65 and over 65 years old, accepted the product over the 80% criteria, whereas the 46-55 years old provided % acceptance was less than 80% (Table 32). Moreover, opinions about flavor differed. The 46-55 year old group wanted to add other flavor to Jiaogulan beverage, but the over 55 year old group did not want to add other flavors. Therefore, the suitable target group for this product should be consumers over 55 years old. This finding generally agrees with Fanelli and Stevenhagen (1976) who reported that the diet of older adults tends to be less varied than that of younger adults. This change in likes, called sensory-specific satiety (Rolls, 1986) was one of the reasons the elderly diet may be more monotonous the elderly have diminished sensory-specific satiety (Popper and Kroll, 2003). The data analysis showed the different needs of consumer groups regarding beverage types. The younger group (age under 55 years old)

preferred the ready to drink beverage but the older group needed the powder infusion beverage. So, the product profile for this target consumer (age over 55 years old) is the tea powder in tea bag infusion in 100 ml of hot water with the natural flavor.

In conclusion, the likely development of Jiaogulan beverage will be in the powder infusion beverage and the tea bag form for the target consumers over 55 years old.

Table 31 Mean hedonic rating^{1, 2} by age

Attributes	Age (years)			
	35 – 45	46 – 55	56 – 65	> 65
Color ^{ns}	5.85±1.99	6.06±1.94	6.44±1.69	6.14±2.11
Aroma ^{ns}	6.15±1.65	6.22±1.81	6.33±1.60	6.37±1.94
Flavor	5.89±1.82 ^b	6.22±1.87 ^{ab}	6.55±1.76 ^a	6.69±1.77 ^a
Overall liking	6.02±1.81 ^b	6.28±1.95 ^{ab}	6.64±1.47 ^a	6.61±1.83 ^a

n=416

¹ Ratings are based on a 9-point hedonic scale 1 = dislike extremely, 5 = neither like nor dislike and 9 = like extremely (Peryam and Pilgrim, 1957).

² Means within same row not followed by the same letters are significantly different ($p \leq 0.05$) as determined by Duncan New Multiple Rank Test (DMRT) means separation test.

ns = Not significantly different ($p > 0.05$) as determined by Duncan New Multiple Range Test (DMRT) means separation test.

Table 32 Cross tabulations between age and acceptance, between age and flavor added and between age and beverage form preferences of the respondents

(n=416)

Attributes	Age (years)			
	35 – 45	46 – 55	56 – 65	> 65
<u>Acceptance</u>				
$(\chi^2=19.92^*)^2$				
Accept	77 (70.0%) ³	82 (77.4%)	86 (85.1%)	88 (88.9%)
Reject	33 (30.0%)	24 (22.6)	15 (14.9%)	11 (11.1%)
<u>Flavor</u>				
<u>adding</u> $(\chi^2=22.25^*)$				
Yes	73 (66.4%)	66 (62.3%)	43 (42.6%)	40 (53.4%)
No	37 (33.6%)	40 (37.7%)	58 (57.4%)	59 (59.6%)
<u>Beverage form</u>				
$(\chi^2=38.28^*)$				
Dried leaf	19 (17.3%)	22 (20.8%)	29 (28.7%)	34 (34.3%)
Powder in bag	29 (26.4%)	32 (30.2%)	35 (34.7%)	44 (44.4%)
Instant powder	11 (10.0%)	14 (13.2%)	15 (14.9%)	8 (8.1%)
Ready to drink	51 (46.4%)	38 (35.8%)	22 (21.8%)	13 (13.1%)

¹ Respondents involved in consumer survey in Thailand during January – February , 2004.

² Pearson chi-square are significantly at $p \leq 0.01$.

³ Percentage of respondents in the same age group.

4.4 Process Development of Jiaogulan Tea

4.4.1 Kinetic of Jiaogulan Tea Infusion

Solid extraction yield (SEY) of tea infusion in different temperatures is shown in a typical graph (Figure 32), similar to Liang and Xu (2003) who studied the effect of extraction temperature of black tea. Results of the Jiaogulan tea infusion at various temperatures demonstrate that the SEY rose steeply during the first 10 min of infusion and increased gradually after 20 min. The SEY increased slowly even if the extraction duration was extended to 2 hours. This concentration of the sample was always similar to one taken after only one hour and therefore it was assumed equilibrium had been reached (Smith and Thomas, 2003). These equilibrium values were found to be independent of the temperature extraction. The equilibrium SEY at 60-90°C increased from 384.71 g/kg to 493.26 g/kg (Table 33). The result showed that the SEY of 60°C was not significantly different from 70°C, so the 70°C extraction was selected for the next study. Jaganyi and Mdletshe (2000); Jaganyi and Ndlovu (2001) showed further that a first-order process of caffeine flowed through the tea bag membrane in kinetic equation 2.

$$\ln (C_{\infty} / C_{\infty} - C) = k_{obs} t + a \quad (\text{Equation 2})$$

From the equation when attempting to analyze the data obtained from different temperatures the graph of $\ln (C_{\infty} / C_{\infty} - C)$ versus t was used. This graph should produce a linear plot with first-order rate constant. Figure 33 shows the linear graphs of constant rate of extraction (0-5 min) in different temperatures. And the data of rate constant, and equilibrium concentration is shown in Table 33. It was indicated that the risen temperature (60-90 °C) affected to the rate constant whereas increasing from 3.334 to 6.156×10^{-3} /s. This result was supported by Smith and Thomas (2003) who studied the infusion of coffee soluble into water. The rate of infusion of coffee soluble can be seen to rise steeply with the increase in temperature and there was a relationship between first order rate constants and temperature. Besides, Jaganyi and Mdletshe (2000) reported the effect of tea-bag material on the

rate and temperature of caffeine extraction from black Assam tea. The result indicated that the first order rate constants for the tea-bag tea were found to be 29% smaller than those of loose tea but the rate constant was increased by the rising of the extraction temperature.

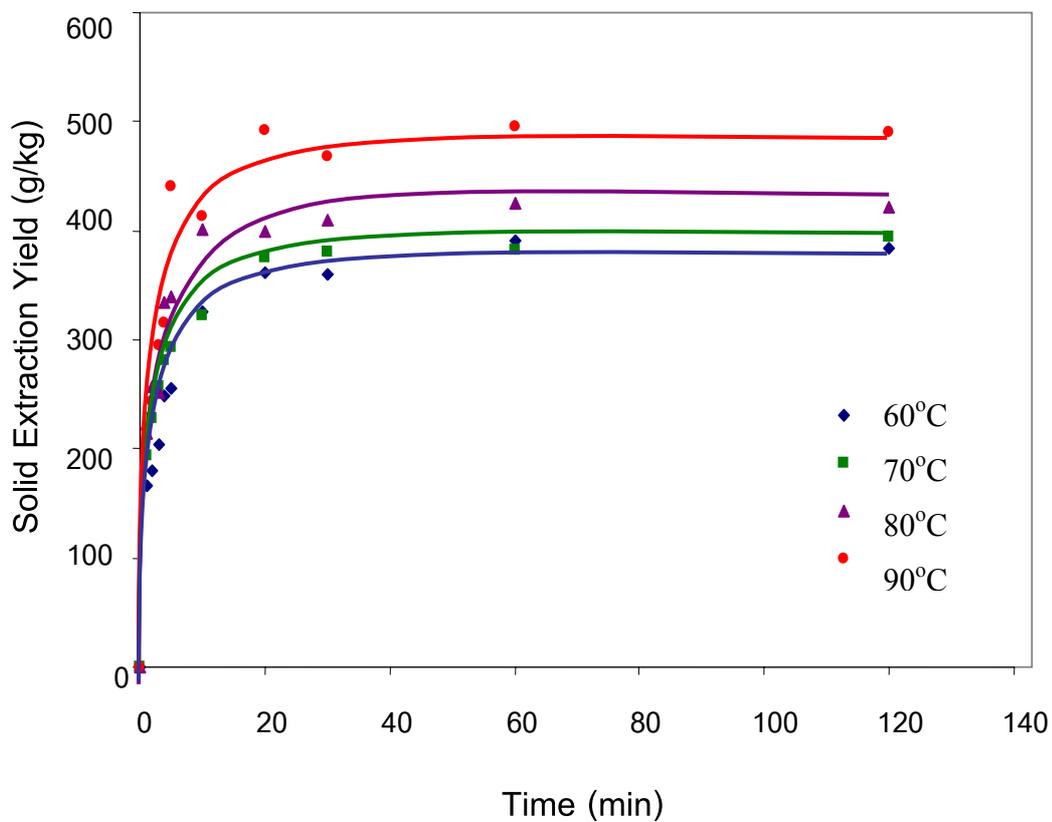


Figure 32 Effect of infusion temperature on extracted solid yield.

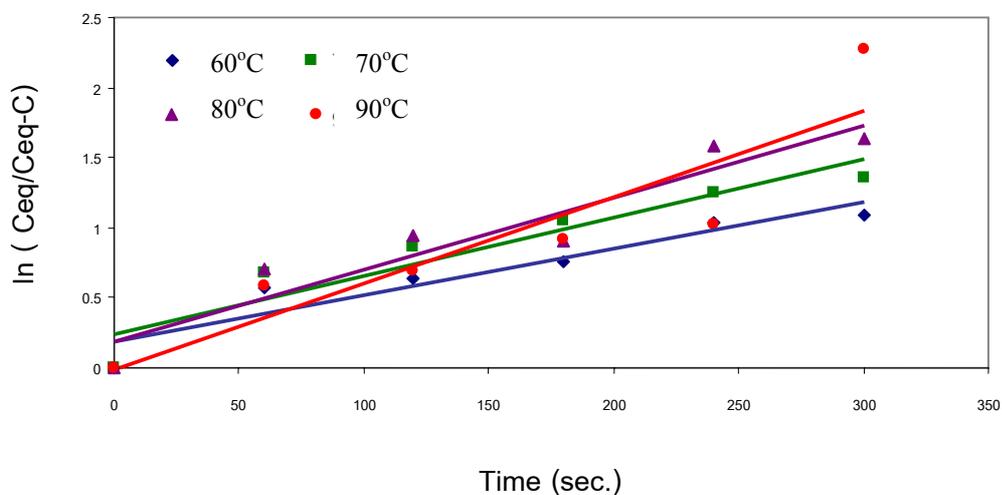


Figure 33 Kinetic plots comparing the increased temperatures.

Table 33 Kinetic and equilibrium data for Jiagulan tea infusion over a temperature range

Temperature (°C)	Rate constant $K_{obs} \cdot 10^{-3} / s$	Intercept A	Equilibrium concentration (C_{∞} , g/kg)
60	3.334	0.18	384.71 ± 1.21^c
70	4.145	0.24	394.44 ± 7.25^c
80	5.137	0.19	442.12 ± 8.52^b
90	6.156	-0.01	493.26 ± 3.01^a

The different letters in the same column mean significantly different ($p \leq 0.05$)

4.2 Effect of Temperature and Time on Jiaogulan Tea Infusion

This experiment was conducted to investigate the effect of temperature and time on chemical qualities of Jiaogulan tea infusion. The temperature was varied in range of 70-80-90°C and time in range of 1-5-10 min. The result of solid extraction yield, antioxidant activity and total saponin content in tea were examined.

The solid extraction yield of tea infusion started from 11.05% to 28.56% (Figure 34). The time factor affected the SEY but the temperature was not significantly different (Table 34). Also, the combination treatments were significantly different for SEY. From the extracted solid yield point of view, extraction at 90°C was ideal for normal hot drinking instant tea (Liang and Xu, 2003).

The total saponin content varied from 160.80 to 585.65 µg /ml (Figure 35). Both time and temperature influenced the saponin content in the tea solution (Table 34). The high temperature increased the extraction of total saponin content in tea solution. According to Jaganyi and Price (1999), temperature (70-90°C) ascended the rate of infusion of caffeine from South Africa black tea. Additionally, the temperature of extraction increased the caffeine in black Assam tea (Jaganyi and Mdletshe, 2000).

The antioxidant activity increased from 325.90 to 843.55 µg Trolox/ml (Figure 36). Likewise for saponin, both time and temperature affected the antioxidant activity in the tea solution (Table 34). The result indicated the extraction at 90°C for 10 min provided the highest TEAC.

In this experiment, the results suggested that the optimum extraction should be at 90°C for 10 min because the maximum of saponin and antioxidant properties were realized at this temperature and time.

Table 34 Effect of time and temperature on SEY, total saponin and antioxidant activity in Jiaogulan tea infusion

Source of variance	F-value (P)		
	SEY	Total saponin	TEAC
Temperature	0.778 (0.488)	5.752* (0.025)	6.191* (0.020)
Time	14.34* (0.002)	10.873* (0.004)	24.506* (0.000)
Temperature x Time	0.611 (0.665)	0.613 (0.665)	3.442 (0.057)

* means significant difference at $p \leq 0.05$

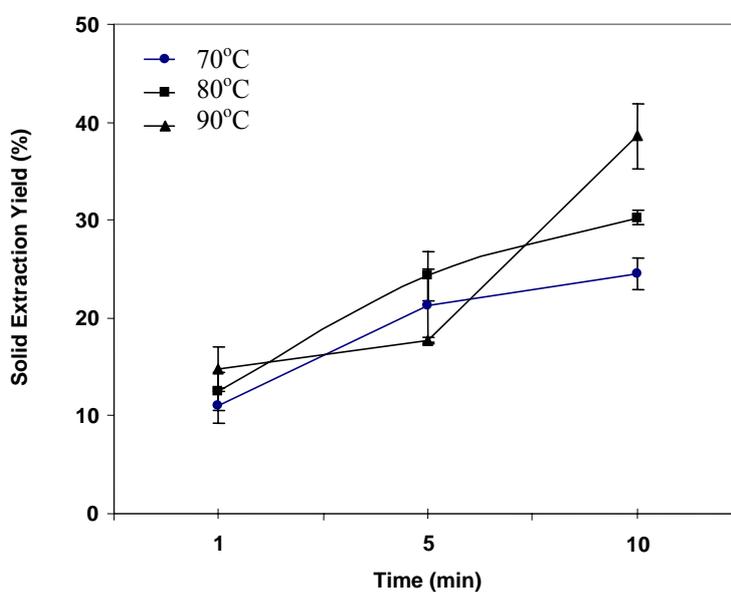


Figure 34 The solid extraction yield of tea infusion at different times and temperatures.

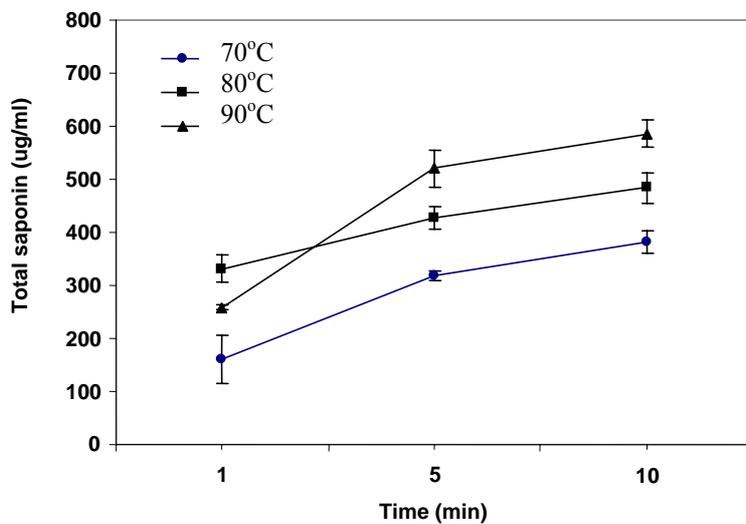


Figure 35 The total saponin yield of tea infusion at different times and temperatures.

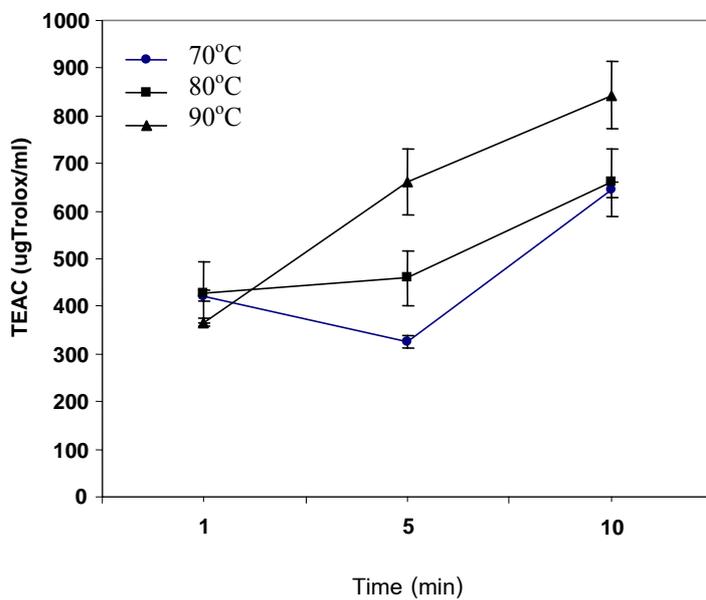


Figure 36 The antioxidant activity yield of tea infusion at different times and temperatures.

4.5 Optimization of Saponin in Jiaogulan Tea

Six treatment combinations in Table 6 were employed in a pentagon design which showed varied saponin content (0-500 mg) in Jiaogulan tea and quantity of water (50-250 ml) for infusion at 90°C for 10 minutes. The objective was to optimize the chemical compositions and sensory properties for meeting the acceptability of consumers. The data were collected in mean and standard deviation from the Jiaogulan tea infusion in the chemical data, sensory descriptive data and hedonic rating data. All attributes are significantly different among six treatments.

4.5.1 Chemical Properties

The main constituents of Jiaogulan were the triterpene saponins as a series of dammarane-type saponin called gypenoside (Piacente and Pizza, 1995; Hu *et al.*, 1997; Liu *et al.*, 2004). The total saponin and antioxidant activity of Jiaogulan tea infusion were significantly different (Table 35). Regression analysis (Table 32) shows the predictive models of total crude saponin and antioxidant activity in Jiaogulan tea solution when R^2 are 0.8294 and 0.9696, respectively. Joglekar and May (1987) suggested that for a good fit of a model, R^2 should be at least 0.80. It means that the model can be used for predicting the response. Therefore, the models of saponin and antioxidant could predict the total saponin content and antioxidant activity in Jiaogulan tea. The total saponin content in Jiaogulan tea infusion increased when the water quantity was reduced (Figure 36(a)) and as the concentration of saponin got higher nearly 0.1 mg/ml. of tea solution. The soluble solid including saponin could be diffused in the water phase by a hot water infusion. Similarly, the antioxidant activity (Figure 36(b)) of the tea solution increased when the saponin content was high in concentration. This is on the principle of diffusion; the transfer of solutes from a solid to a fluid state is used to extract sugar, pectin, coffee and tea (Schwartzberg, 1982). From the study of Jaganyi and Ndlovu (2001) on the kinetics of tea infusion, the results indicated that the shape of a tea bag had no influence of the rate of infusion but the tea bag membrane offered some hindrance to the infusion of caffeine. Likewise, the saponin and other components in Jiaogulan tea can be infused

through the tea bag, although the tea-bag material slowed the infusion of tea (Jaganyi and Mdletshe, 2000).

Table 35 Total saponin content and antioxidant activity of Jiaogulan tea infusion

Treatment ¹	Total saponin (mg/ml)	TEAC (μ g Trolox/ml)
1	0.354 \pm 0.16 ^b	144.65 \pm 4.68 ^a
2	0.180 \pm 0.02 ^b	27.11 \pm 4.20 ^b
3	0.077 \pm 0.03 ^b	19.30 \pm 1.24 ^b
4	0.177 \pm 0.01 ^b	38.67 \pm 10.48 ^b
5	0.841 \pm 0.13 ^a	160.92 \pm 19.36 ^a
6	0.280 \pm 0.05 ^b	64.53 \pm 23.29 ^b

¹ Treatments are the pentagon design points from Table 6.

The different letters in the same column mean significantly different ($p \leq 0.05$).

Table 36 The regression models of the chemical component of Jiaogulan tea infusion

Chemical properties	Regression model	R ²
Chemical component		
Total saponin	0.32 + 0.19 X ₁ - 0.28 X ₂ - 0.23 X ₁ X ₂	0.8294
Antioxidant activity	75.86 + 62.34 X ₁ - 55.46 X ₂ - 48.19 X ₁ X ₂	0.9696

Independent variables are X₁ = saponin content in dried Jiaogulan and X₂ = water.

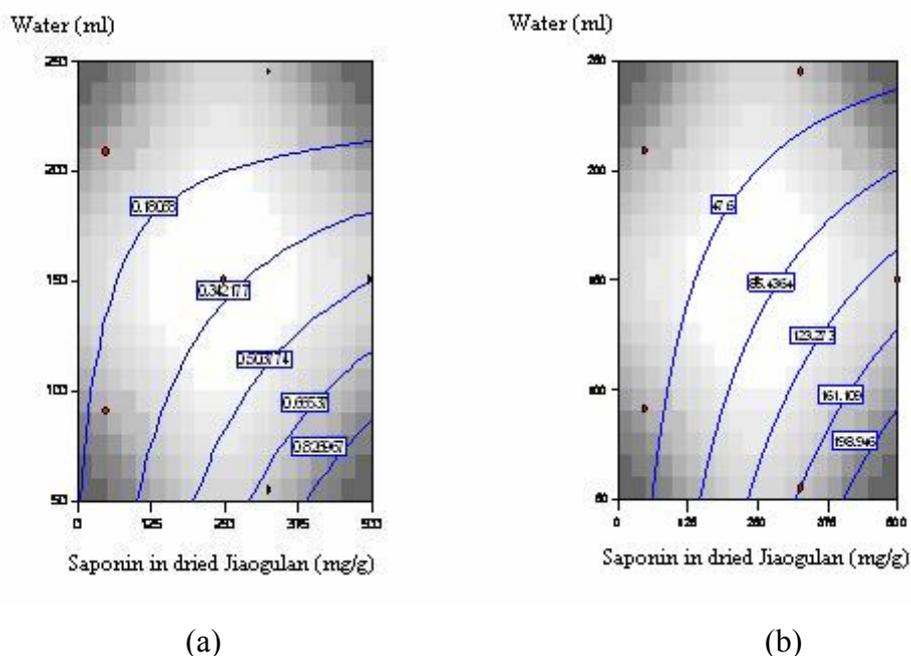


Figure 37 Contour plots of the chemical component in Jiaogulan tea infusion (total saponin (a), antioxidant activity(b)).

4.5.2 Sensory Descriptive Analysis of Jiaogulan Tea Infusion

Twelve trained panelists developed the 13 terms of Jiaogulan tea based on the perception of the following attributes: appearance, aroma, flavor, feeling factor and aftertaste (Table 7). Table 37 shows the attributes of Jiaogulan tea which consists of 2 attributes of appearance (yellowish green color and clearness), 3 attributes of aroma (dried leaf aroma, green tea aroma, Jiaogulan aroma), 2 attributes of tastes (sweet and bitter), 2 attributes of flavors (green tea flavor and Jiaogulan flavor), 1 attribute of feeling factor (astringency), and 3 attributes of aftertaste (sweet, bitter and astringent aftertaste). The consensus and intensity of the references by trained panels is shown in Table 8. The appearance of Jiaogulan tea was in yellowish green in color because of the chlorophyll pigment. The color scale was from light yellow to dark green. Yau and Huang (2000) reported that the clearness is an important attribute of Oolong tea which is the same as Jiaogulan tea but the sedimentation is not a requirement of tea infusion. Baykan (1981) pointed that the aroma is one of the most important criteria of tea. The aromatic attribute of Jiaogulan tea consists of specific Jiaogulan aroma and green tea aroma. It contains dried leaf

aroma, such as dried grass or dried rice leaf or dried bamboo leaf, which may get in during the drying process of Jiaogulan tea. The astringency attribute is one of the tea characteristics of green tea and black tea, as well as Jiaogulan tea. The bitter taste of Jiaogulan is from its saponin glycoside content (Cheeke, 2001), which is the active compound in *Gynostemma pentaphyllum* (Hu *et al.*, 1966; Cui *et al.*, 1999) and the bitter taste still endures as an aftertaste. Normally, the glycosides structure is connected to a sugar molecule. The glycosides of terpenoids or steroids are a variety of sweet-tasting natural products (Nishizawa and Yamada, 1996). For this reason, the panel could detect the sweet taste and sweet aftertaste of Jiaogulan tea that is different from other tea products.

Regression analysis of the sensory attributes of Jiaogulan tea and the R^2 values are listed in Table 38. The coefficient of determination, R^2 , is defined as the ratio of the explained variation to the total variation and is a measure of the degree of fitness (Haber and Runyon, 1977). It is also the proportion of the variability in the response variables, which is accounted for by the regression analysis (Mclaren *et al.*, 1977). Henika (1982) explained that variances above 85% are sufficient for sensory data. Therefore, all regression models were used to predict the responses of sensory descriptive attributes of Jiaogulan tea because the R^2 values are greater than 0.85. The results show that the saponin content increases all sensory attributes. On the other hand, the water could decrease the intensity of all sensory attributes because the amount of water could dilute the intensity of saponin.

Table 37 Sensory descriptive mean values of Jiaogulan tea infusion

Treatments ¹	Color	Clearness	Dried leaf aroma	Green tea aroma	Jiaogulan aroma	Sweet	Bitter
1	81.54±12.16 ^{ab}	60.28±14.62 ^{ab}	39.71±8.18 ^{ab}	47.29±9.45 ^a	72.75±8.56 ^{ab}	36.14±11.93 ^a	65.29±11.55 ^a
2	38.93±17.50 ^c	23.25±22.57 ^c	23.36±17.39 ^c	23.79±9.96 ^c	38.89±12.28 ^c	17.36±8.44 ^b	23.5±13.33 ^c
3	39.43±19.59 ^c	23.0±22.46 ^c	21.75±13.97 ^c	21.89±11.31 ^c	37.43±14.36 ^c	16.0±11.92 ^b	19.57±12.77 ^c
4	70.0±15.17 ^b	49.29±18.75 ^b	33.93±5.38 ^b	41.07±8.59 ^{ab}	66.25±8.78 ^{ab}	36.0±6.75 ^a	51.25±6.38 ^b
5	86.64±12.34 ^a	69.29±19.05 ^a	44.25±7.49 ^a	48.79±10.61 ^a	75.0±6.86 ^a	38.0±12.13 ^a	69.14±10.24 ^a
6	67.46±8.88 ^b	44.86±5.79 ^b	36.57±8.59 ^{ab}	36.61±8.59 ^{ab}	62.64±10.25 ^b	34.29±7.11 ^a	54.25±7.01 ^b

Table 37 (Continued)

Treatments ¹	Green tea flavor	Jiaogulan flavor	Astringency	Sweet aftertaste	Bitter aftertaste	Astringent aftertaste
1	34.89±5.35 ^a	70.89±6.07 ^a	54.89±9.47 ^a	26.62±9.49 ^a	75.5±9.80 ^a	66.39±8.78 ^{ab}
2	19.50±5.49 ^b	36.68±10.94 ^b	19.86±9.56 ^c	10.36±5.80 ^b	24.07±8.21 ^c	25.64±15.08 ^d
3	15.74±9.75 ^b	30.86±14.67 ^b	15.89±9.23 ^c	8.89±6.33 ^b	19.96±9.60 ^c	25.29±17.48 ^d
4	31.11±5.95 ^a	66.29±8.12 ^a	43.82±8.89 ^b	26.50±8.81 ^a	58.39±10.03 ^b	53.25±9.74 ^c
5	36.36±9.16 ^a	72.5±10.9 ^a	57.36±10.31 ^a	29.43±8.81 ^a	77.36±12.11 ^a	69.04±11.83 ^a
6	31.64±3.89 ^a	63.94±6.15 ^a	44.25±5.89 ^b	25.57±7.87 ^a	59.07±8.48 ^b	56.07±6.68 ^b

¹ Treatments are the pentagon design points from Table 6.

The different letters in the same column mean significantly different ($p \leq 0.05$).

Table 38 The regression models of sensory descriptive attributes¹ of Jiaogulan tea infusion

Attributes	Regression model ²	R ²
Color	$68.31 + 12.72 X_1 - 25.34 X_2 - 10.34 X_2^2$	0.9993
Clearness	$45.00 + 12.16 X_1 - 23.69 X_2$	0.9856
Dried leaf aroma	$33.26 + 6.25 X_1 - 10.81 X_2$	0.9622
Green tea aroma	$36.57 + 7.51 X_1 - 14.02 X_2$	0.9536
Jiaogulan aroma	$58.83 + 9.63 X_1 - 20.51 X_2$	0.9299
Sweet taste	$29.63 + 4.47 X_1 - 12.55 X_2$	0.8689
Bitter taste	$47.17 + 14.65 X_1 - 24.81 X_2$	0.9435
Green tea flavor	$28.21 + 5.70 X_1 - 10.03 X_2$	0.9174
Jiaogulan flavor	$56.86 + 10.41 X_1 - 21.96 X_2$	0.8874
Astringency	$43.29 + 12.18 X_1 - 20.83 X_2$	0.9453
Sweet aftertaste	$23.23 + 4.11 X_1 - 11.39 X_2$	0.8878
Bitter aftertaste	$58.40 + 17.51 X_1 - 29.40 X_2 - 14.57 X_2^2$	0.9958
Astringent aftertaste	$54.50 + 12.84 X_1 - 23.08 X_2 - 12.52 X_2^2$	0.9976

¹ Sensory descriptive scales are based on 150 mm unstructured line scale (Stone *et al.*, 1980).

² Independent variables are X_1 = saponin in dried Jiaogulan, X_2 = water.

The study of saponin content on sensory properties of Jiaogulan tea infusion is shown by response surface graphs in Figure 38 and 39. The saponin content had the affect of increasing the intensity of all sensory attributes of Jiaogulan tea infusion. For the color and flavor, the solutes transfer from a solid to an adjacent fluid in the leaching process of tea (Schwartzberg, 1980; Schwartzberg and Chao, 1982) to make a higher intensity of color, aroma and flavor with attributes of yellowish green, dry leaf aroma, green tea aroma, Jiaogulan aroma, green tea flavor and Jiaogulan flavor, as well as sweet and bitter taste. Dos *et al.* (2005) investigated the effect of different factors on sensory attributes (odor, taste and pungency), overall acceptance and preference of Rooibos (*Aspalathus linearis*) tea. The results indicated that the odor of the infused tea had a higher intensity than the traditionally brewed tea. Yin *et al.* (2004) analyzed the glycosides from *Gynostemma pentaphyllum* and isolated sugars from aglycone, which consists of glucose, rhamnose, xylose and arabinose. These sugars could diffuse into the drink to make it more sweetness. This study also demonstrates that saponin could provide the bitter taste (Cheeke, 2001) of Jiaogulan tea as well as other food and beverage product. The astringency attribute is influenced by the saponin content. Moreover, the aftertaste is also affected by the saponin content, causing it to have a sweet, bitter or astringent aftertaste. Studying the flavonoid compound in tea by a time-intensity procedure, the maximum bitter intensity decreased whereas the astringency increased (Peleg *et al.*, 1999), that means there was an interaction between bitterness and astringency attributes. But in this study, if the saponin content increases, the bitter taste and astringency attributes also increase.

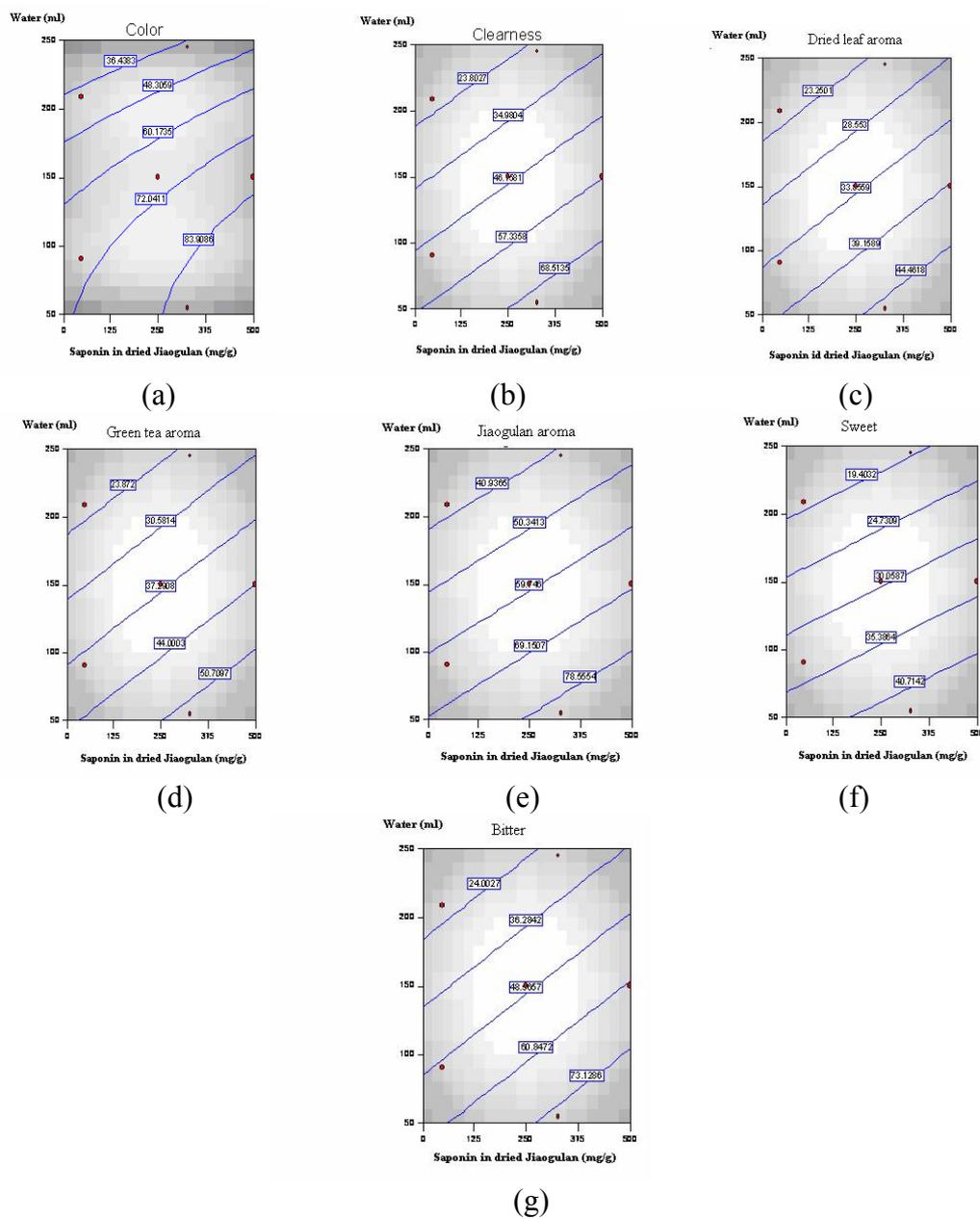


Figure 38 Contour plots of Jiaogulan tea infusion in terms of descriptive attributes: appearance, aroma and taste (color (a), clearness (b), dried leaf aroma (c), green tea aroma (d), Jiaogulan aroma (e), sweet (f) and bitter (g)).

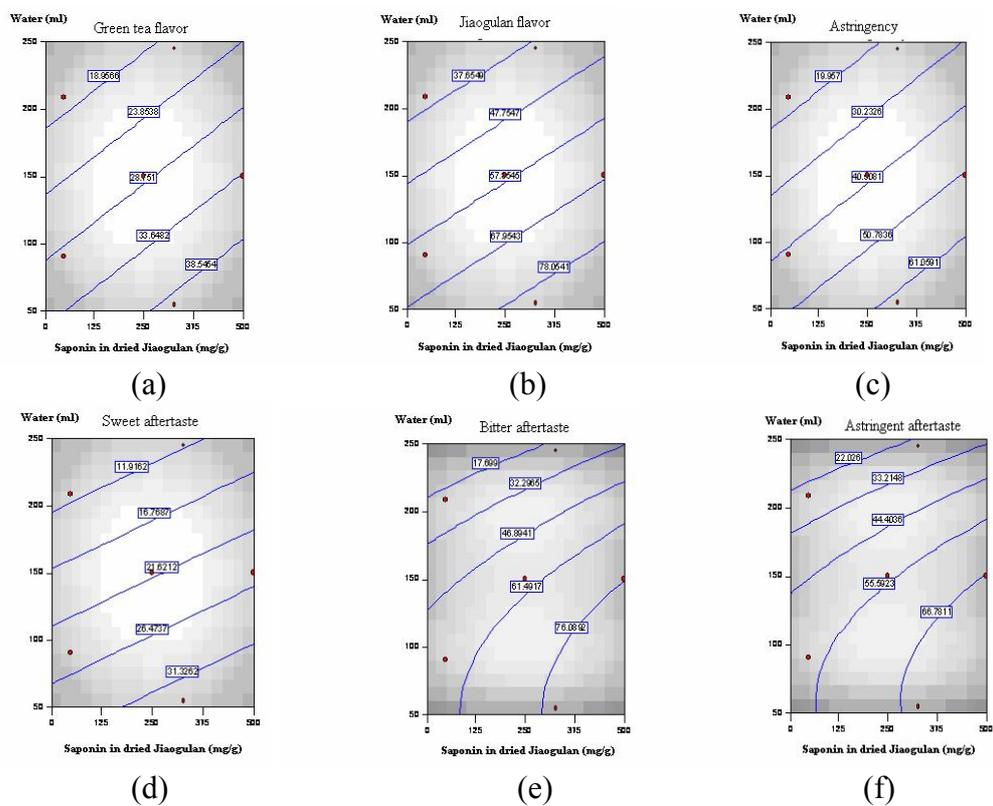


Figure 39 Contour plots of Jiaogulan tea infusion in terms of descriptive attributes: flavor, feeling factor and aftertaste (green tea flavor (a) and Jiaogulan flavor(b), astringency (c), sweet aftertaste (d), bitter aftertaste (e), astringent aftertaste (f)).

4.5.3 Hedonic Rating of Jiaogulan Tea Infusion

The mean hedonic ratings of six Jiaogulan tea treatments were significantly different (Table 39). Table 32 shows the list of regression equations and their corresponding R^2 values which are more than 0.85 (Henika, 1982) except aroma rating. The regressions showed that the saponin content reduced the acceptability of Jiaogulan tea. But the water content could increase the acceptability score of overall liking, taste rating and aftertaste rating because the amount of water could dilute the intensity of taste and aftertaste that increase the acceptance of this product. There was an interaction terms between saponin and water content. The response surface graphs of hedonic rating are shown in Figure 40. The overall liking, color, aroma, taste and aftertaste rating scores had increased when the saponin content was lower. The reason might be due to the intensity of the bitter taste, astringency and aftertaste which increases when saponin was high in content. Mattes (1994) reported that bitter-tasting foods and beverages generally are disliked by most human, such as foods with a prominent bitter taste constitute a very small proportion of humanity's total consumption. But the factors that would underlie a possible reversal of the innate rejection of bitter taste and the rated shift in hedonic response to bitter foods and beverage remain unclear (Stein *et al.*, 2003). Mattes (1994) described variations in the influence of the factors between individuals and products that may explain the differences in an individual's acceptability and use of foods and beverages containing alcohol, caffeine and other bitter compounds. Akella *et al.* (1997) reported that rate of acceptance of green tea decreases as bitterness increases. Stein *et al.* (2003) suggested that repeated exposure can enhance a hedonic evaluation of a bittersweet beverage, perhaps through a learned association of flavor with post-ingestive consequences. Maile and Heymann (1998) evaluated the changes of flavored and herbal teas stored at different temperatures over 11 months and concluded that although the descriptive analysis of the panel indicated overall changes in the products, the consumers either did not detect or care about the changes. Mostly, the hedonic score of overall liking, color and aroma rating of Jiaogulan tea is higher than the 6 point. This means that consumers accepted the overall, color and aroma of this product (Resurreccion, 1998; Grosso and Resurreccion, 2002).

Table 39 Mean hedonic rating of Jiaogulan infusion treatments by consumer

Treatments ¹	Overall liking	Color	Aroma (ns)	Taste	Aftertaste
1	5.83±1.84 ^b	6.13±1.88 ^{ab}	6.00±1.70	5.81±1.83 ^{ab}	5.88±1.94 ^{ab}
2	6.15±1.56 ^{ab}	6.08±1.53 ^b	6.31±1.34	6.02±1.62 ^{ab}	6.08±1.77 ^{ab}
3	6.23±1.60 ^{ab}	6.27±1.62 ^{ab}	6.08±1.61	6.25±1.54 ^{ab}	6.35±1.47 ^{ab}
4	6.73±1.20 ^a	6.96±1.29 ^a	6.63±1.33	6.63±1.36 ^a	6.71±1.25 ^a
5	5.77±2.03 ^b	6.25±1.86 ^{ab}	6.18±1.57	5.42±2.23 ^b	5.63±2.15 ^b
6	6.48±1.54 ^{ab}	6.58±1.54 ^{ab}	6.69±1.17	6.42±1.66 ^a	6.50±1.53 ^{ab}

(n=50)

¹ Treatments are the pentagon design points from Table 6.The different letters in the same column mean significant difference ($p \leq 0.05$).**Table 40** The regression models of consumer acceptance¹ of Jiaogulan tea infusion

Attributes	Regression model ²	R ²
Overall liking	$6.15 - 0.39 X_1 + 0.0027 X_2 + 0.56 X_1 X_2$	0.9705
Color	$6.38 - 0.31 X_1 - 0.23 X_2 + 0.44 X_1 X_2$	0.8717
Aroma	$6.46 - 0.17 X_1 - 0.082 X_2 + 0.47 X_1 X_2 - 0.34 X_2^2$	0.7847
Taste	$6.31 - 0.43 X_1 + 0.14 X_2 + 0.57 X_1 X_2 - 0.52 X_2^2$	0.9780
Aftertaste	$6.38 - 0.43 X_1 + 0.091 X_2 + 0.48 X_1 X_2 - 0.45 X_2^2$	0.9696

¹ Consumer acceptance ratings are based on a 9-point hedonic scale, 1=dislike extremely, 5=neither like nor dislike and 9=like extremely (Peryam and Pilgrim, 1957).² Independent variables are X_1 = saponin content in dried Jiaogulan, X_2 = water.

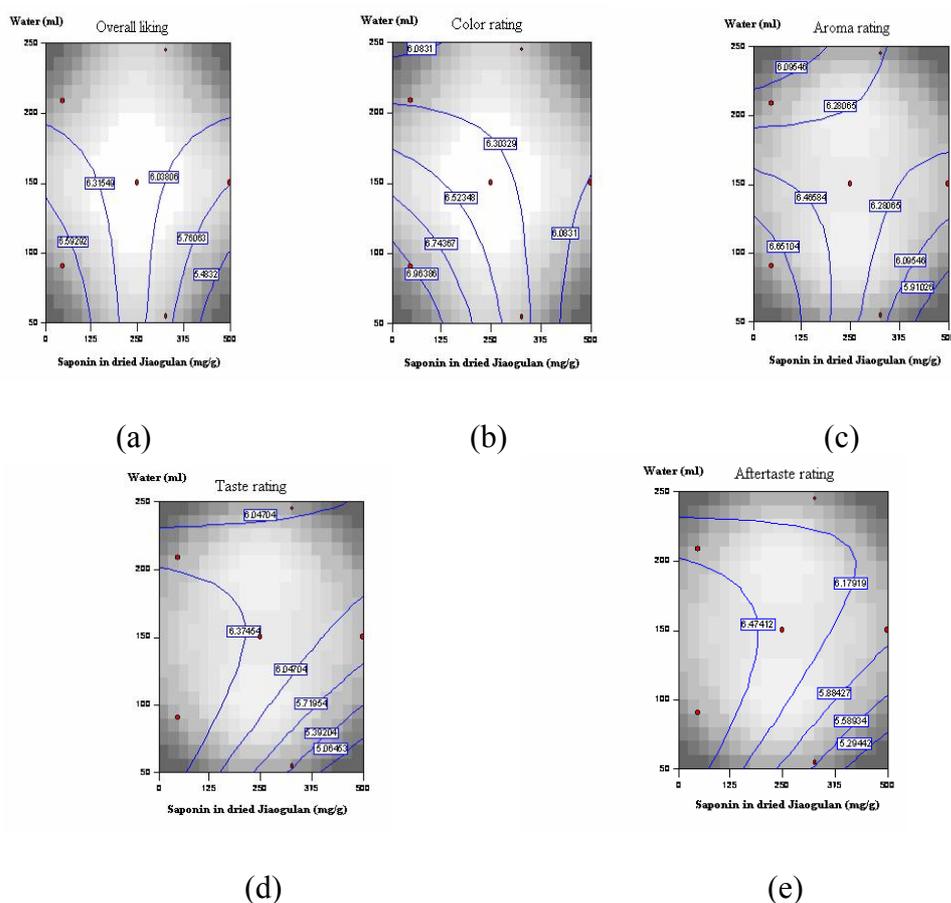


Figure 40. Contour plots of hedonic rating of Jiaogulan tea infusion (overall liking (a), color (b), aroma (c), taste (d), aftertaste (e)).

4.5.4 The Optimization

The optimization technique provides product formulation for consumer evaluation (Gacula, 1993). The optimum formula was determined by superimposing the contour plots of all the responses (Sin *et al.*, 2005). The optimum contour map of Jiaogulan tea for sensory descriptive attribute and hedonic rating over 6.0 (Resurreccion, 1998, Grosso and Resurreccion, 2002) was overlaid. The concentration of saponin in tea infusion that consumers like was not too high. This means that the saponin content must be brewed in a large quantity of water. However, as we need this product to contain the maximum active components, so the optimum area should contain the highest saponin content and highest antioxidant

activity. Therefore, by overlaying all the responses, the optimum combined condition shows in Figure 41. The optimum Jiaogulan tea formula consisted of the dried Jigogulan containing 235-310 mg of total saponin infused in hot water (80-115 ml). Furthermore, the computer generated the optimized point for saponin and water at 292 mg and 100 ml, respectively. The consumer survey showed the target consumers liked to drink 100 ml of beverage. This tea infusion could be the optimum of active components that consumers would accept in terms of its sensory properties.

Table 41 shows the predicted values of chemical components, sensory descriptive and hedonic rating from optimum saponin and water. The total saponin in Jiaogulan tea infusion was 0.50 mg/ml and the antioxidant activity was 112.63 μg Trolox/ml. The hedonic ratings of overall liking, color, aroma, flavor and aftertaste rating were 6.12, 6.50, 6.50, 6.0 and 6.13, respectively.

In conclusion, Jiaogulan tea can be described in terms of its 13 attributes by a sensory descriptive analysis. These attributes include: yellowish green color, clearness, dried leaf aroma, green tea aroma, Jiaogulan aroma, sweet taste, bitter taste, green tea flavor, Jiaogulan flavor, astringency, sweet aftertaste, bitter aftertaste and astringent aftertaste. The consumer acceptability test indicated that they liked this tea if the saponin content is decreased. The optimization of the maximum chemical components overlaid with the acceptability score (> 6) which is in the area of 235-310 mg saponin in dried Jiaogulan and 80-115 ml water. Finally, this study suggests that the optimum formulation of Jiaogulan tea contains 292 mg of saponin in dried Jiaogulan and 100 ml of hot water, in order for consumers to accept this tea.

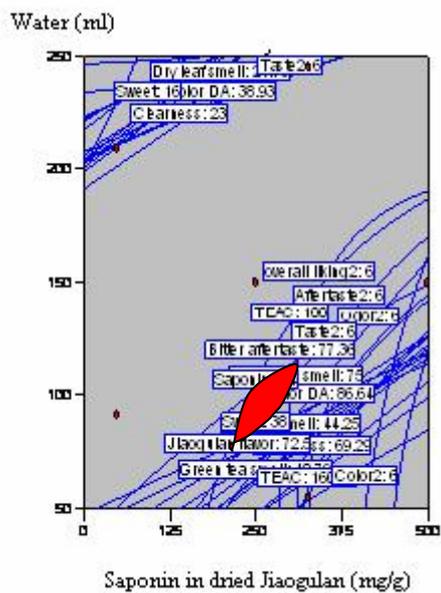


Figure 41 The optimization of sensory descriptive, consumer acceptance test and chemical component of Jiaogulan tea infusion.

Table 41 Predicted values of Jiaogulan tea from regression models.

Attributes of Jiaogulan tea infusion	Predicted value
Chemical component	
Total saponin ($\mu\text{g/ml}$)	0.50
Antioxidant activity (mg Trolox/ml)	112.63
Sensory descriptive attributes ¹	
Color	87.84
Clearness	60.03
Dried leaf aroma	40.14
Green tea aroma	45.47
Jiaogulan aroma	71.82
Sweet taste	37.55
Bitter taste	62.95
Green tea flavor	34.58
Jiaogulan flavor	70.77
Astringency	52.60
Sweet aftertaste	28.41
Bitter aftertaste	71.48
Astringent aftertaste	64.33
Hedonic rating ²	
Overall liking	6.12
Color rating	6.50
Aroma rating	6.50
Flavor rating	6.00
Aftertaste rating	6.13

¹ Sensory descriptive scales are based on 150 mm unstructured line scale (Stone *et al.*, 1980).

² Hedonic ratings are based on a 9-point hedonic scale; 1=dislike extremely, 5=neither like nor dislike and 9=like extremely (Peryam and Pilgrim, 1957).

4.6 Finished Product Qualities of Jiaogulan Tea

The Jiaogulan tea was a fine particle of dried Jiaogulan leaves (1.80 g) in a tea bag. The finished product qualities are shown in Table 42. The developed Jiaogulan tea consists of 6.55% moisture, 12.5% total ash, 9.52% insoluble ash and 26.92% solid extraction. The active compound was 161.68 mg per gram of total saponin, so the tea bag contained 292 mg of total saponin. Compared to the standard of Jiaogulan development set by the Medicinal Plant Research Institute, Department of Medical Science, Ministry of Public Health, Thailand (Medicinal Plant Research Institute, 2005), the developed Jiaogulan tea qualities are above the standard (Table 41). The moisture content and total ash are less than 8% and 14%, respectively. The solid extraction yield is greater than 21%, especially total saponin is two time more than the standard. The nutritional quality of Jiaogulan tea sample consists of 4.65% protein, 3.32% fat and 12.13% crude fibre, as well as, the antioxidant activity which is 57.57 mg Trolox per gram. The color of the tea powder was the same green color as other green tea products, which provided L^* value (35.06), a^* value (-0.13) and b^* (19.05). After brewing the Jiaogulan tea bag in 90°C water for 10 min, the tea solution consisted of total saponin (0.496 mg/ml) and antioxidant activity (105.8 µg Trolox/ml). The water activity of dried Jiaogulan tea is 0.40, so no pathogenic bacterial can grow in this product. Likewise, the microbial qualities of Jiaogulan tea product are in the microorganism standard (Table 41), including, total aerobic microbial count, yeast and moulds, *Escherichia coli*, *Staphylococcus aureus*, *Clostridium* spp., and *Salmonella* spp. Therefore, this Jiaogulan tea is high in quality for target consumers.

Table 42 Finished product quality of Jiaogulang tea

Quality	Jiaogulan Tea (%)	Standard Jiaogulan ¹ (%)
Moisture content (%)	6.55±0.03	≤8.0
Total solid (%)	93.45±0.03	>92.0
Total ash (%)	12.50±0.24	≤14.0
Insoluble ash (%)	9.52±0.23	-
Soluble ash (%)	2.98±0.01	-
Solid extraction by hot water (%)	26.92±3.82	≥21.0
Protein (%)	4.65±0.25	-
Fat (%)	3.32±0.02	-
Crude fiber (%)	12.13±2.39	-
Total saponin (mg/g)	161.68±5.57	≥80.0
Total antioxidant activity (mg Trolox/g)	57.57±2.59	-
L*	35.06±0.50	-
a*	-0.13±0.08	-
b*	19.05±0.51	-
Water activity	0.40	-
Total aerobic microbial count (cfu/g)	4.81x10 ⁴	<5x10 ⁵
Yeast and Moulds (cfu/g)	2.15x10 ³	<5x10 ³
<i>Escherichia coli</i> (cfu/g)	<10	<50
<i>Staphulococcus aureus</i>	No	No
<i>Clostridium spp.</i>	No	No
<i>Salmonella spp</i>	No	No

¹ Standard of airy *Gynostemma pentaphyllum* (Thumb.) Makino sitting by the Medicinal Plant Research Institute, Department of Medical Science, Ministry of Public Health, Thailand.

4.7 Consumer Acceptance Test of Jiaogulan Tea (n=200)

The consumer acceptance test of developed Jiaogulan tea was conducted by 200 respondents who lived in Bangkok (n=91) and Chiangmai (n=109), Thailand. Resurreccion (1998) stated that acceptance tests give an estimate of product acceptance in different areas around the country and the participants may be composed of 100-500 consumers. The demographic profile of the respondents is shown in Table 43. There were 67% females and 33% males whose ages were 55-82 years old. Almost 50% were retired adults (49%), housewives (27.5%) and business owners (14%). The incomes of the respondents were less than 5,000 baht/month (27%) and more than 30,000 baht/month (14.5%). The education was divided into 5 levels: primary school (24.5%), high school (22.5%), vocational degree (11%), bachelor degree (28%) and advance degree (14%). More than half of respondents always exercises 3-6 times a week (51%) and daily exercise (28%).

Table 43 The demographic profile of the respondents¹ in consumer test

(n=200)			
Respondent characteristic	Response (%)	Respondent characteristic	Response (%)
Gender		Education	
Female	67.0	Bachelor degree	28.0
Male	33.0	Primary school	24.5
		High school	22.5
		Advance degree	14.0
		Vocational degree	11.0
Employment		Income	
Retirement	49.0	(baht/month)	
Housewives	27.5	< 5,000	27.0
Business owner	14.0	5,001 – 10,000	10.5
Government worker	4.0	10,001 – 15,000	16.0
Employee	1.0	15,001 – 20,000	7.5
Others	4.5	20,001 – 25,000	13.5
		25,001 – 30,000	11.0
		> 30,000	14.5

¹Respondents involved in the consumer acceptance test in Bangkok and Chiangmai, Thailand during December, 2005 – January, 2006.

Mean hedonic rating of two hundred consumers for Jiaogulan tea is presented in Table 44. Overall acceptability of the product was rated at like moderately range (7.02), where as the color rating was 7.17 as well as aftertaste rating (7.07). The other attributes were rated between like slightly to like moderately such as odor rating (6.96), overall flavor rating (6.90), sweetness rating (6.47) and bitterness rating (6.42). Comparison to Bangkok and Chiangmai, the hedonic ratings of Chiangmai are higher than Bangkok. However, Resurreccion (1998) pointed that hedonic rate should be more than 6 for acceptance of food products (Grosso and

Resurreccion, 2002). Likewise, Dos *et al.* (2005) evaluated the Rooibos tea by consumers, the overall acceptability was moderately liked with a score between 6 and 8.2 on the 11-point scale. Therefore, this Jiaogulan tea was accepted by the target consumer. Confirmation was obtained by the acceptance question, 92% of consumer accepted this product (Table 44) by itself that means it has a high potential in the market.

Table 44 Mean hedonic rating¹ of Jiaogulan tea as rate by target consumers from different locations

Attributes rating	Locations		Average (n=200)
	Bangkok (n=91)	Chiangmai (n=109)	
Overall liking	6.85±1.06 ^b	7.17±0.96 ^a	7.02±1.02
Color ^{ns}	7.09±1.12	7.23±1.13	7.17±1.12
Aroma ^{ns}	6.84±1.17	7.06±1.08	6.96±1.12
Overall flavor ^{ns}	6.81±1.27	6.97±1.16	6.90±1.21
Sweetness	6.16±1.78 ^b	6.72±1.13 ^a	6.47±1.52
Bitterness ^{ns}	6.33±1.64	6.49±1.41	6.42±1.52
Aftertaste ^{ns}	7.01±1.26	7.12±1.19	7.07±1.22

¹ Ratings are based on a 9-point hedonic scale 1 = dislike extremely, 5 = neither like nor dislike and 9 = like extremely (Peryam and Pilgrim, 1957).

ns = non significant difference (p>0.05).

The different letters in the same row compared between Bangkok and Chiangmai mean significantly different (p≤0.05).

Discriminant analysis was used to predict the acceptability of a given sample through the calculation of posterior probabilities of membership to either the acceptable or unacceptable group (Frank *et al.*, 1990). Discriminant analysis predicted the acceptance group and the unacceptance group of Jiaogulan tea with 91% hitting rate. The important factors were overall liking and sweetness rating which affect the acceptability of this product. Likewise, discriminant technique could be used to predict the buying decision group of Jiaogulan tea. More than half of consumers (69%) were willing to buy this product but 31% of consumer decided not to buy (Table 45). The result shows the discriminant functions of the buying group (D_1) and the not buying group (D_2) with group centroids. The Fisher discriminant function ($D_1 - D_2$) could predict buying decision of other consumers, if they had tested Jiaogulan tea with 71% hitting rate. Discriminant analysis technique was used to determine which set of sensory variables discriminated one group of objects from another (Resurreccion, 1988). Furthermore, the standardized canonical discriminant function indicated that the hedonic rating for sweetness and aftertaste were the important factors which influence the buying decisions of consumers. Therefore, the producers of Jiaogulan tea product should concern about sweetness and aftertaste of product that affected consumer acceptance and achieved on selling this product.

Table 45 Percent of acceptance and buying decision by consumer

	(n=200)	
Acceptance	Accept	Reject
Location ($\chi^2=0.142$, ns)		
Bangkok	41.5%	4%
Chiangmai	50.5%	4%
Total	92%	8%
Buying decision	Buy	Not buy
Location ($\chi^2=3.16$, ns)		
Bangkok	28.5%	17%
Chiangmai	40.5%	14%
Total	69%	31%

Discriminant analysis of acceptance

Discriminant function of a accept group:

$$D_1 = -46.904 + 5.641 \text{ Overall liking} + 2.339 \text{ Color rating} \\ + 2.272 \text{ Aroma rating} + 1.396 \text{ Overall flavor rating} \\ + 0.582 \text{ Sweetness rating} + 0.042 \text{ Bitterness rating} \\ + 0.671 \text{ Aftertaste rating} \quad (\text{group centroid} = 0.208)$$

Discriminant function of a reject group:

$$D_1 = -27.224 + 3.858 \text{ Overall liking} + 2.557 \text{ Color rating} \\ + 1.924 \text{ Aroma rating} + 0.802 \text{ Overall flavor rating} \\ + 0.039 \text{ Sweetness rating} - 0.028 \text{ Bitterness rating} \\ + 0.545 \text{ Aftertaste rating} \quad (\text{group centroid} = -0.604)$$

Fisher discriminant function:

$$D_1 - D_2 = -6.043 + 0.021 \text{ Overall liking} + 0.08 \text{ Color rating} \\ + 0.141 \text{ Aroma rating} + 0.93 \text{ Overall flavor rating} \\ + 0.021 \text{ Sweetness rating} - 0.056 \text{ Bitterness rating} \\ + 0.433 \text{ Aftertaste rating}$$

Standardized discriminant function:

$$D = 0.594 \text{ Overall liking} - 0.091 \text{ Color rating} \\ + 0.139 \text{ Aroma rating} + 0.195 \text{ Overall flavor rating} \\ + 0.299 \text{ Sweetness rating} + 0.053 \text{ Bitterness rating} \\ + 0.101 \text{ Aftertaste rating}$$

Hitting rate = 91%

Discriminant analysis of buying decision

Discriminant function of a buying group:

$$D_1 = -38.037 + 3.539 \text{ Overall liking} + 2.75 \text{ Color rating} \\ + 2.065 \text{ Aroma rating} + 0.719 \text{ Overall flavor rating} \\ + 0.317 \text{ Sweetness rating} + 0.076 \text{ Bitterness rating} \\ + 0.889 \text{ Aftertaste rating} \quad (\text{group centroid} = 0.272)$$

Discriminant function of a not buying group:

$$\begin{aligned}
 D_1 &= -31.994 + 3.518 \text{ Overall liking} + 2.647 \text{ Color rating} \\
 &+ 1.924 \text{ Aroma rating} + 0.802 \text{ Overall flavor rating} \\
 &+ 0.039 \text{ Sweetness rating} - 0.028 \text{ Bitterness rating} \\
 &+ 0.545 \text{ Aftertaste rating} \quad (\text{group centroid} = -0.604)
 \end{aligned}$$

Fisher discriminant function:

$$\begin{aligned}
 D_1 - D_2 &= -6.043 + 0.021 \text{ Overall liking} + 0.08 \text{ Color rating} \\
 &+ 0.141 \text{ Aroma rating} - 0.083 \text{ Overall flavor rating} \\
 &+ 0.278 \text{ Sweetness rating} + 0.104 \text{ Bitterness rating} \\
 &+ 0.344 \text{ Aftertaste rating}
 \end{aligned}$$

Standardized discriminant function:

$$\begin{aligned}
 D &= 0.023 \text{ Overall liking} + 0.129 \text{ Color rating} \\
 &+ 0.176 \text{ Aroma rating} - 0.11 \text{ Overall flavor rating} \\
 &+ 0.46 \text{ **Sweetness rating**} + 0.174 \text{ Bitterness rating} \\
 &+ 0.455 \text{ **Aftertaste rating**}
 \end{aligned}$$

Hitting rate = 71%

The effect of the health benefit information and the composition of Jiaogulan tea caused some consumers to change their mind. Firstly, 62 respondents were not willing to buy this product but after receiving the information about the Jiaogulan research, 20 of them changed their minds and bought this product (Table 46). The McNemar test indicated that there was significantly difference ($p \leq 0.05$). The 95% CI for the difference of proportions is [0.059, 0.141]. It means that the probability that consumers would buy the Jiaogulan tea after given the facts is 0.059-0.141 times higher than the probability that consumers would buy it before informed about the product health benefits. The reason for the change of mind was they thought that this product would benefit their health. According to the Lappalainen *et al.*(1998) survey, it was found that health is one of the frequently mentioned motivations behind food choices. Likewise, Dos *et al.* (2005) researched the

acceptability of Rooibos tea. After the product testing was completed, the panelists were informed of the general health effects of Rooibos tea and its composition, and 89% of the panelists mentioned that they would buy the product, which might be an indication of health consciousness. Finally, most consumers (90%) thought the optimum price of Jiaogulan tea is 5 Baht per tea bag which will influence them to buy this product.

Table 46 The buying decision of consumer before and after knowing the product information

Before knowing the product information	After knowing the product information		Total	χ^2	95%CI ¹
	Yes	No			
Yes	138	0	138		
No	20	42	62	20.0	(0.059, 0.141)
Total	158	42	200	(3.84) ²	

¹ CI means confidence interval of 95% different proportions.

² Chi-square value from chi-square table at $\alpha = 0.05$, d.f. = 1.

4.8 Effect of Storage Temperature on Stability of Saponin and Antioxidant Activity

This experiment was conducted to investigate the stability of saponin and antioxidant activity in Jiaogulan tea during 4 months in the United States. Jiaogulan was packed in tea bags and kept in 3 storage conditions; 35°C, 45°C and ambient temperature of 23-25°C. The main chemical components, total saponin and antioxidant activity, were measured every month. The saponin content at the initial time was at 72.86±7.58 mg/g. The total saponin content during the four months is

shown in Figure 42. There is not significantly different found in the saponin between the temperatures in the time duration. The quantity was varied from 58.89 to 77.46 mg/g. The storage temperature did not affect saponin content. Bobeyko and Kintia (1996) reported that all the classes of steroidal glycoside and saponins are thermostable up to 150-170°C. Therefore, saponin is a heat resistant substance, so it is a stable compound in a stored condition. Likewise, the antioxidant activity was similar to saponin. The antioxidant status during 4 months is shown in Figure 43. There are no significant differences of total antioxidant status in Jiagulan tea, whereas the TEAC started at 21.09 to 24.62 mg Trolox/g at fourth month. Naturally, the most pronounced change resulted from oxidation reactions occurring rapidly in heating or slowly in storage. The transformation of antioxidants, such as glycosides into aglycones, might increase resistance against oxidation during processing and storage (Pokorny and Schmidt, 2001). As a result, the antioxidant and saponin content were stable in the Jiagulan tea during the 4 month period.

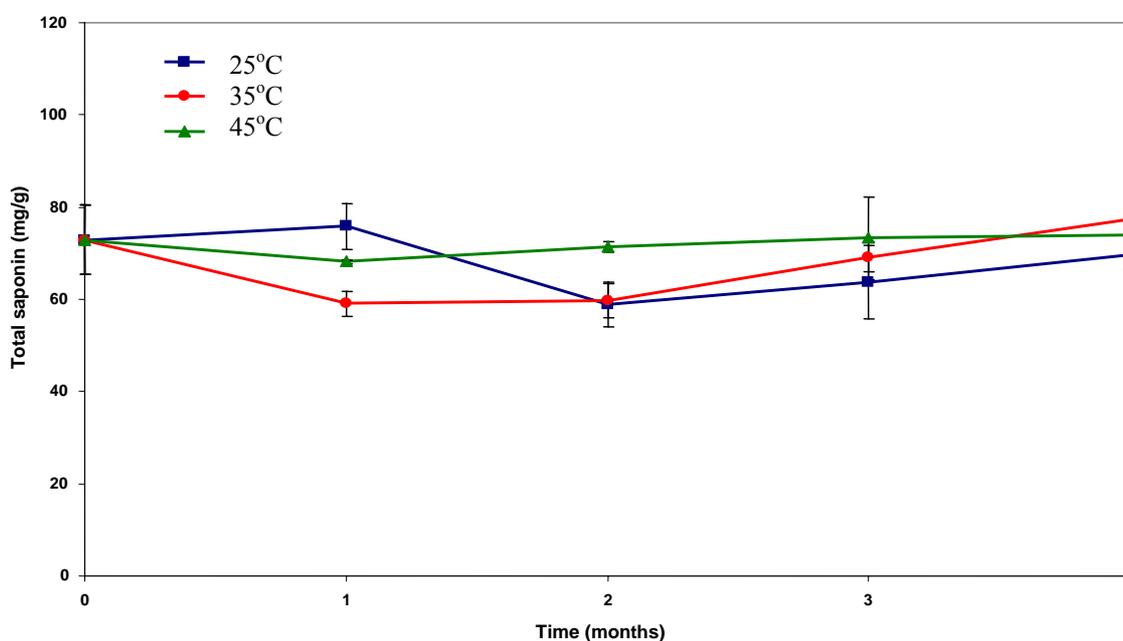


Figure 42 The total saponin content in Jiaogulan tea during storage time.

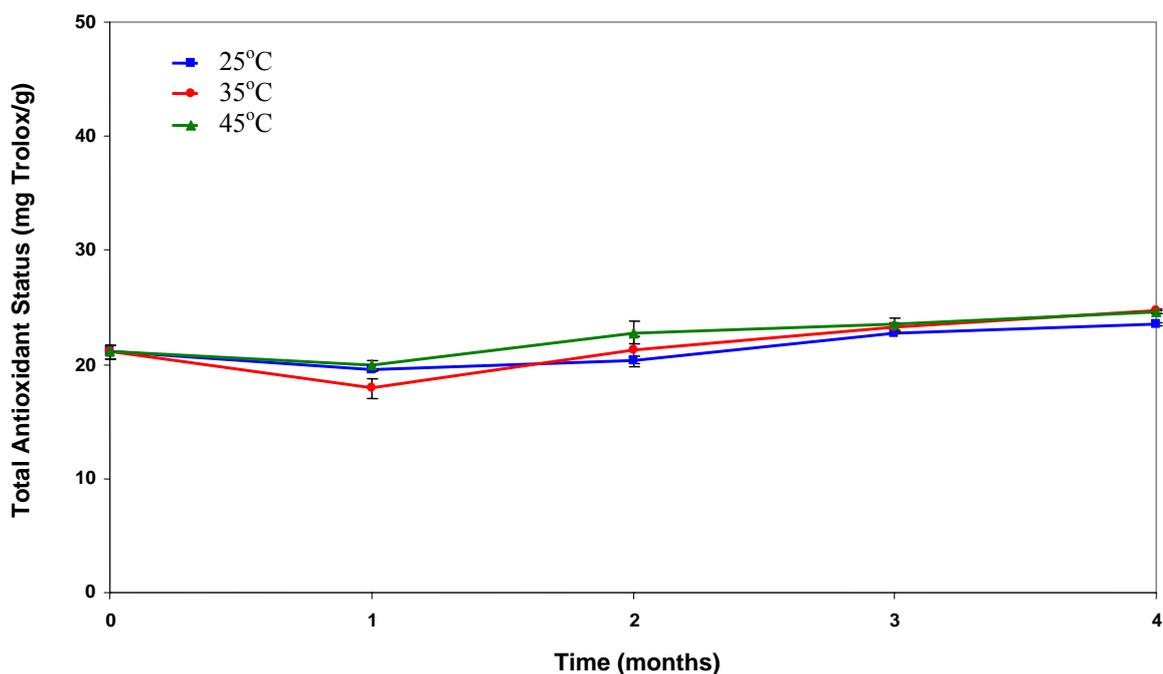


Figure 43 Total antioxidant status in Jiaogulan tea during storage time.

4.9 Product Specification

4.9.1 Product Description

Product name: Jiaogulan tea

The tea is made from Jiaogulan leaves (*Gynostemma pentaphyllum*) which are a natural flavor. It has no foreign matter and no foreign flavor. The dried powder tea was packed into the tea bag at 1.80 g minimum. Normally, the product is brewed in the hot water (90°C) for 10 min. The tea solution consists of natural flavor, saponin and antioxidants which provide health benefits. Drinking Jiaogulan tea continuously could reduce triglyceride and lower total cholesterol levels.

4.9.2 Product Specification

The Jiaogulan tea product was processed from Jiaogulan leave (*Gynostemma pentaphyllum*). The raw material was Jiaogulan plants age at least 5 months old. Jaiogulan herb consists of saponin glycosides (called gypenoside) which are the same compounds as ginsenoside Rb1 and Rg1 from ginseng (*Penax* spp.). This tea contains at least 292 mg of saponin. Jiaogulan leaves were washed, cleaned, vacuum microwave dried and ground to a fine particle. The moisture content is below 8% and $a_w = 0.4$. All product specifications which include raw material specification, process control and packaging specification are shown in Table 47.

Table 47 The specification of Jiaogulan tea product

	Specification
Product Specification	<ul style="list-style-type: none"> - Processed from good quality of Jiaogulan leave and no foreign matter. - The dried powder product packed into the tea bag at 1.80 g - Moisture content < 8% and Water activity (a_w) < 0.4 - Total saponin content at least 80 mg/g
Raw Material Specification	<ul style="list-style-type: none"> - Jiaogulan herb (<i>Gynostemma pentaphyllum</i>) with 5 months old - The herb was harvested and trimmed by hand for the good quality of leaves and processed at the same day. - The Jiaogulan leave consist of total saponin > 80 mg/g
Process Control Specification	<ul style="list-style-type: none"> - The raw material was cleaned and washed to remove the foreign matter. - Drying process with the vacuum microwave dryer at 2400 watt for 25 min for 500 g of raw material. - Grinding into a fine particle at 0.30-0.85 mm. particle size - Packing in the tea bag at 1.80 g - Brewing method was sleep in 90°C hot water for 10 min

Table 47 (continued)

	Specification
Quality Control	- Moisture content of Jiaogulan tea < 8.0%
Specification	- Total ash < 14%
	- Solid extraction yield > 21%
	- Water activity < 0.40
	- Total saponin > 80.0 mg/g
	- Total antioxidant activity > 50.00 mg Trolox/g
	- Total aerobic microbial count < 5×10^5 cfu/g
	- Yeast and Moulds < 5×10^3 cfu/g
	- <i>E. coli</i> < 50 cfu/g
	- Sensory descriptive quality of Jiaogulan tea infusion
Packaging	- The tea bag was made from white tea bag paper
Specification	- The tea bag size was 4.0 cm. width x 6.0 cm. long
	- The small pack was 10 tea bags in paper box
	- The large pack was 25 tea bags in paper box

4.10 Cost of Jiaogulan Tea Product

Cost of the Jiaogulan tea product included raw material cost, packaging cost, and cost accounting. The raw material cost was 70 Baht/g of Fresh Jiaogulan plant. In the process, Jiaogulan leaf yield was 62.95% and dried Jiaogulan yield was 9.70%. The mass balance of Jiaogulan drying is shown in Figure 44. The product cost was calculated as follow:

The raw material cost :

Fresh Jiaogulan plant	70 Baht/kg
Fresh Jiaogulan leaves	111.20 Baht/kg
Dried Jiaogulan	1,146.39 Baht/kg

The product cost of tea bag

1 tea bag = 1.80 g of dried Jiaogulan	2.06 Baht/ tea bag
The labor cost (20%)	0.41 Baht/ tea bag
The packaging cost	0.50 Baht/ tea bag
The total cost	2.97 Baht/ tea bag

In conclusion, the total cost of Jiaogulan tea was 2.97 Baht/ tea bag.

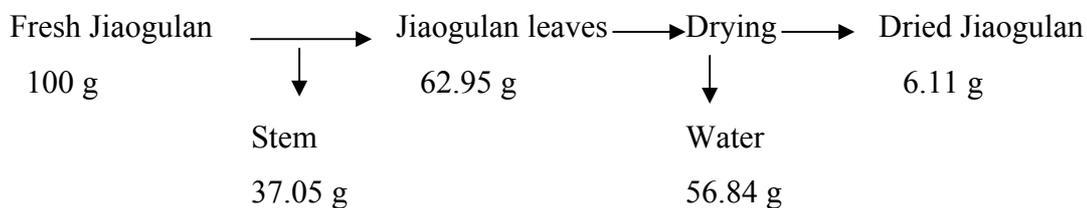


Figure 44 Mass balance of Jiaogulan drying.

CONCLUSION

The aim of this project was to develop a herbal beverage from the Jiaogulan herb for consumers who wanted to maintain their cholesterol and triglyceride levels. The study was conducted in four phases; raw material assessment, drying process of Jiaogulan, identification and effect of Jiaogulan extract in animal model and product development of herbal beverage from Jiaogulan. The brief results are as follow.

1. The study on raw material indicated that the Jiaogulan leaf contained more saponin (204.67 mg/g) and antioxidant activity (23.04 mg Trolox/g) than the stem which contained 105.19 mg/g of saponin and TEAC 5.26 mg Trolox/g. So, only the Jiaogulan leaf was selected to be the raw material for this product.

2. The raw material was prepared by two drying processes; hot air drying and vacuum microwave drying with 10% final moisture controlling. The drying curves showed that vacuum microwave drying process took only a short time compared to hot air drying. The total saponin contents were not significantly different, where the values were 94.9-155.51 mg/g. In contrast, the antioxidant activity was significantly difference ($p \leq 0.05$), which microwave dried Jiaogulan contained 37.98-56.72 mg Trolox/g but hot air drying reduced the antioxidant properties to 4.48-7.71 mg Trolox/g. Thus, the vacuum microwave was better than the hot air drier. The optimum condition of vacuum-microwave was to use 2400 watt for 25 min which produced the highest qualities.

3. The identification of Jiaogulan water extract compared to methanol and ethanol extraction were conducted by GC-MS. The GC-MS chromatogram showed 3 aglycones of gypenosides which were extracted by methanol and ethanol, whereas Jiaogulan water extract consisted of 2 aglycones. Mass spectrum was matched with ginsenoside Rb1 which was the 20(S)-dammar-24-ene-3 β , 12 β , 20 triol, and the other compound was 20(S)-dammar-24-ene-3 β , 6 α , 12 β , 20 tetrol as ginsenoside Rg1. While, the alcoholic solvents could extract one more peak with a structure that consisted of CH₂OH. The toxicity of Jiaogulan was investigated in rats. The acute

test during 14 days showed that there was no toxic sign and the severity. The LD₅₀ of Jiaogulan extract was > 32 g/kg (or > 80.36 g/kg dried Jiaogulan). So, Jiaogulan tea was shown to be non toxic and safe for consumers. Furthermore, the Jiaogulan water extract was studied for the effect on serum lipids in hypercholesterolemia rats. Ten rats were fed with a basal diet for 10 days, and then fed with a high cholesterol diet (1% cholesterol) for 14 days, where the cholesterol levels were increasingly significant. After being fed 3% Jiaogulan extract for 2 weeks, the results showed that Jiaogulan extract could significantly reduce triacylglycerol levels and lower cholesterol levels.

4. The main phase was the development of herbal beverage from dried Jiaogulan. Six focus groups discussed their opinions about herbal beverages and commented on the Jiaogulan tea product. As qualitative research, the consumer survey was conducted in four big cities in Thailand; Bangkok, Chiangmai, Khonkean and Songkha. 416 respondents, whose age range was divided into four groups; 35-45, 46-55, 56-65 and over 65 years old, participated in this study. The results showed consumer attitudes and behavior concerning herbal beverages. The factor analysis indicated that the most salient factor for consumers was the sensory factor rather than other factors; the belief factor, the information of product, the herb type, the marketing factor and the consumption factor. The binary logistic regression detected the sensory factors which affected the acceptability of Jiaogulan tea. These were taste and overall liking. The target consumer could be the age 55 years old because they accepted this product more than younger consumers. Finally, the product profile was the Jiaogulan powder in the tea bag with natural flavor.

5. The study was the process development of Jiaogulan tea infusion. It was found that an infusion temperature of 90°C for brewing for 10 min could extract the maximum saponin and antioxidant properties. The optimization of saponin in Jiaogulan tea was conducted for saponin content and water for sensory properties and chemical components and consumer acceptance. The sensory descriptive analysis by trained panelists defined 13 attributes of Jiaogulan tea; yellowish green, clearness, dried leaf aroma, green tea aroma, Jiaogulan aroma, sweet, bitter, astringency, sweet

aftertaste, bitter aftertaste and astringent aftertaste. The response surface graphs were superimposed with sensory descriptive attributes, maximum chemical properties and a hedonic rating over 6 (n=50). The optimum Jiaogulan tea consisted of 292 mg of saponin with 100 ml of water.

6. The finished product qualities were 6.55% moisture, 12.50% total ash, 26.92% extract solid, 161.68 mg saponin/g and TEAC 57.57 mg Trolox/g. Additionally, the total quality of Jiaogulan tea was in accordance with the Thai herbal standard.

7. The confirmation study was a consumer acceptance test of 200 target consumers (> 55 years old). The hedonic ratings of overall liking, color, aroma, overall flavor, sweetness, bitterness and aftertaste were 7.02, 7.17, 6.96, 6.47, 6.42 and 7.07, respectively. For the acceptance question, 92% of respondents accepted this tea. Furthermore, the McNemar test indicated that the health benefit information could change buying decisions of consumers.

8. Final study, the effect of storage temperature on the active compounds showed that there was no significant differences for saponin content and antioxidant activity during 4 months of storage.

RECOMMENDATION FOR FUTURE WORK

The recommendation for future work would be the raw material quality control in order to control total saponin in Jiaogulan plant.

The drying process was vacuum-microwave drying using small amount of Jiaogulan leaves. Scale-up production is essential to confirm the Jiaogulan tea processing as industrial product.

The shelf-life would be studied to predict the shelf-life of the Jiaogulan tea for commercial product.

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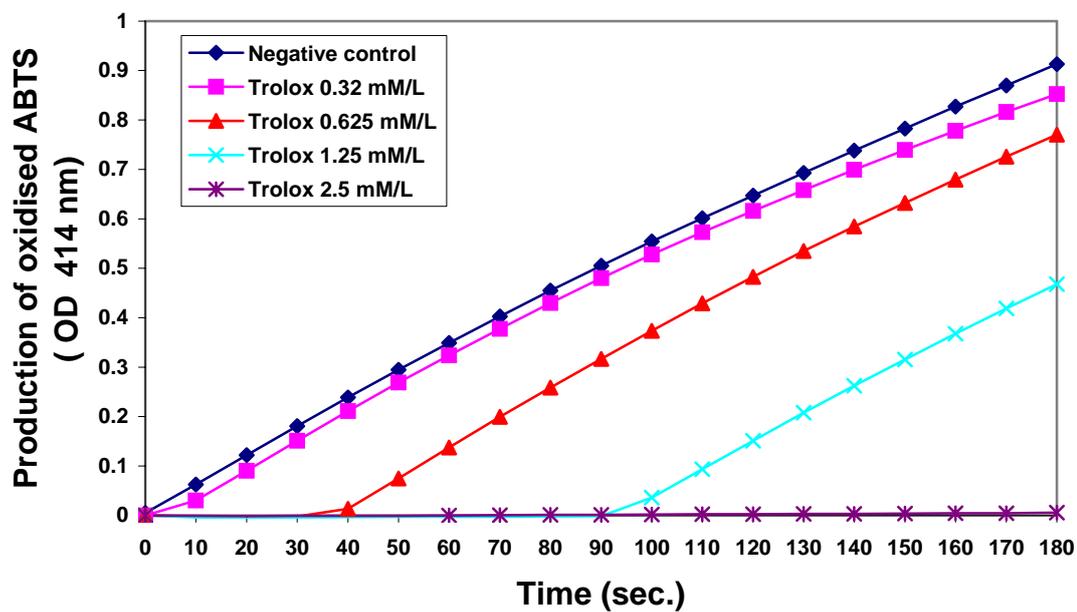
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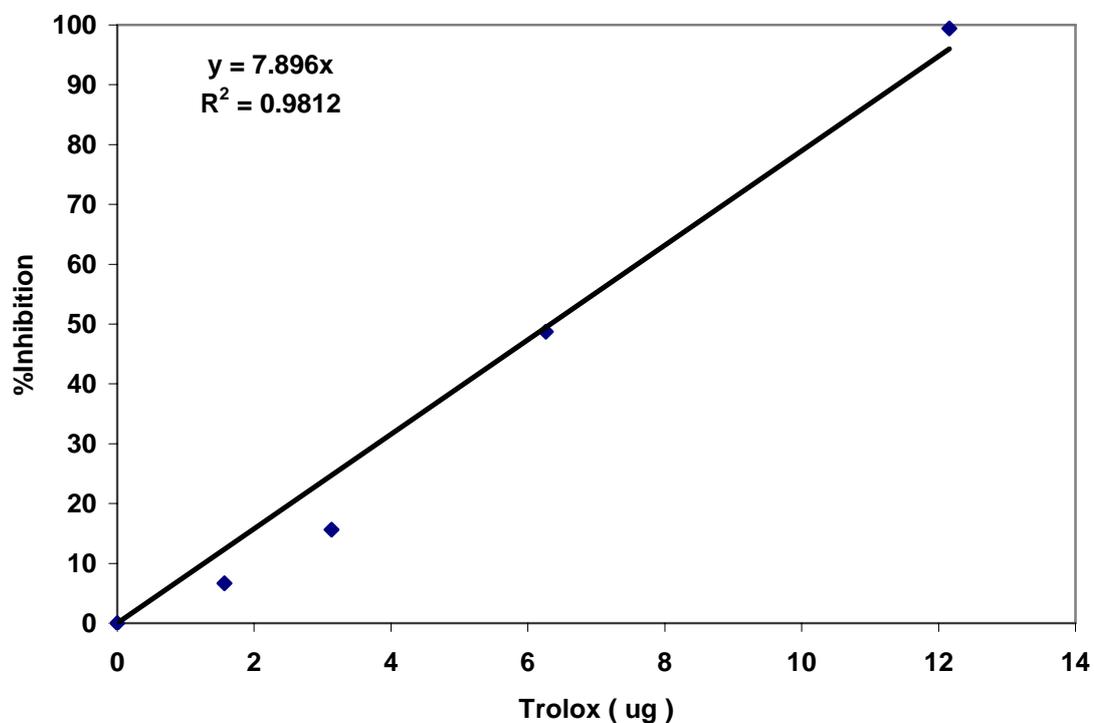
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[Abstract]

APPENDIX 1

Standard Curve of Trolox



Appendix Figure 1 The production of oxidized ABTS by standard Trolox at different concentrations.



Appendix Figure 2 Standard curve of Trolox at different concentrations.

APPENDIX 2

Mineral and Vitamin Mix for Rat Diets

Appendix Table 1 Mineral Mix # 210025 (Dyets Inc., Bethlehem, PA., U.S.A.)

Mineral	Amount (mg/kg diet)
Calcium carbonate	357,000
Potassium phosphate, monobasic	196,000
Potassium citrate.H ₂ O	70,780
Sodium chloride	74,000
Potassium sulfate	46,600
Magnesium oxide	24,000
Ferric citrate, U.S.P.	6,060
Zinc carbonate	1,650
Manganous carbonate	630
Cupric carbonate	300
Potassium iodate	10
Sodium selenate	10.25
Ammonium paramolybdate.4H ₂ O	7.95
Sodium metasilicate.9H ₂ O	1,450
Chromium potassium sulfate.12H ₂ O	275
Lithium chloride	17.4
Boric acid	81.5
Sodium fluoride	63.5
Nickel carbonate	31.8
Ammonium vanadate	6.6
Sucrose, finely powdered	221,026

Appendix Table 2 Vitamin Mix # 310025 (Dyets Inc., Bethlehem, PA., U.S.A.)

Vitamin	Amount (mg/kg diet)
Thiamine HCl	6
Riboflavin	6
Pyridoxine HCL	7
Niacin	30
Calcium pantothenate	16
Folic acid	2
Biotin	0.2
Cyanocobalamin (B-12) (0.1%)	25
Vitamin A palmitate (500,000 IU/g)	8
Vitamin E acetate (500 IU/g)	150
Vitamin D ₃ (400,000 IU/g)	2.5
Vitamin K ₁ premix (10 mg/g)	0.75

APPENDIX 3

Focus Group Discussion: Moderator's Guide

Moderator's Guide: Focus Group Discussion on Herbal Beverage.

1. Introduction (5 min)

1.1 Moderator's Introduction

“Good Evening, my name is Niramon Utama-ang. I am a Ph.D student in Kasetsart University and my major is Agro-Industry Product Development. I am studying about “Development of Herbal Beverage from Jiaogulan Herb”. Today, I would like to moderate the focus group discussion on herbal beverage. Please participate to this focus group.”

1.2 Objective of This Focus Group

“The objectives of this focus group are to investigate the consumer perception, attitude and behavior on herbal beverage.”

1.3 Ground Rules of the Focus Group Discussion

“This focus group discussion will proceed with all of yours cooperation. Please feel free to talk about your opinion. In the discussion, I have to record by tap recorder. Please tell your name before speaking and speak with loud and clear. No interruption when someone is speaking and avoids side conversations with other participants. At the end, you will receive the gift for your co-operation.”

1.4 Self-introductions

“Please introduce yourself to others. Tell us your name, occupation and hobbies.”

2. Reconnaissance (20 min)

“The discussion is started right now. I will ask the questions, so please answer one by one from left to right.”

Question 1: What is the ‘Herbal beverage’ in your opinion?

Question 2: What are your 3 favorite herbal beverages?

Question 3: How often do you drink these herbal beverages?

Where do you buy this herbal beverages?

3. In-deep Investigation (50 min)

Question 4: What is your purpose for herbal beverage drinking?

Question 5: What do you think about the herbal beverages which can reduce fat and blood cholesterol?

Question 6: To develop the herbal beverage, what herbal beverage product style do you like? What about the sensory quality?

Question 7: What packaging do you like for herbal beverage?

Question 8: After testing sample of herbal beverage, what are your opinions about color, odor, taste and others?

4. Closure (5 min)

“Finally, I would like to thank you so much for your cooperation. Please take the souvenir. Thank you very much.”

APPENDIX 4

Questionnaire for Consumer Survey

Questionnaire for Consumer Survey (in Thai)

แบบสอบถาม

เรื่อง การสำรวจความต้องการของผู้บริโภคต่อผลิตภัณฑ์เครื่องตีผสมนไฟร

เรียน ท่านผู้ตอบแบบสอบถาม

แบบสอบถามนี้เป็นการสำรวจความต้องการของผู้บริโภคที่มีต่อผลิตภัณฑ์เครื่องตีผสมนไฟร เพื่อ ใช้ประกอบการเรียบเรียงวิทยานิพนธ์ของ **นางนิรมล อุดมอ่าง** นักศึกษาระดับปริญญาเอก สาขาวิชา **พัฒนาผลิตภัณฑ์อุตสาหกรรมเกษตร มหาวิทยาลัยเกษตรศาสตร์** ภายใต้การดูแลของ **รศ.ดร.เพ็ญขวัญ ชมปรีดา** โดยได้รับทุนสนับสนุนงานวิจัยจาก **มูลนิธิโครงการหลวง** ซึ่งผู้วิจัยใคร่ขอความกรุณาและ ความร่วมมือจากท่านในการตอบแบบสอบถามให้สมบูรณ์

โดยแบบสอบถามแบ่งเป็น 3 ส่วน ดังนี้

ส่วนที่ 1 ข้อมูลด้านทัศนคติ และพฤติกรรมการบริโภคเครื่องตีผสมนไฟร

ส่วนที่ 2 ข้อมูลด้านการพัฒนาผลิตภัณฑ์เครื่องตีผสมนไฟรตัวอย่าง โดยมีการทดสอบชิมตัวอย่าง

ส่วนที่ 3 ข้อมูลทั่วไปของผู้ตอบแบบสอบถาม

ทั้งนี้ข้อมูลทั้งหมดที่ท่านตอบมา จะเป็นประโยชน์อย่างยิ่งสำหรับงานวิจัยดังกล่าว

ขอขอบคุณทุกท่านที่เสียสละเวลาในการตอบแบบสอบถามในครั้งนี้เป็นอย่างสูง

ผู้วิจัย

แบบสอบถาม

เรื่อง การสำรวจความต้องการของผู้บริโภคต่อการพัฒนาผลิตภัณฑ์เครื่องต้มสมุนไพร

เฉพาะเจ้าหน้าที่

ส่วนที่ 1 ข้อมูลด้านทัศนคติและพฤติกรรมการบริโภคเครื่องต้มสมุนไพร

1. เครื่องต้มสมุนไพรที่ท่านชอบต้ม โดยเลือกเพียง 3 ชนิดเท่านั้น

กรุณาเรียงลำดับความชอบ 1-3 โดย 1 = ชอบมากที่สุด และ 3 = ชอบน้อยที่สุด

- | | | |
|-----------------------|--------------------|-----------------|
| () ชาเขียว/ชาญี่ปุ่น | () ชาจีน | () น้ำขิง |
| () น้ำเก๊กฮวย | () น้ำกระเจี๊ยบ | () น้ำมะตูม |
| () น้ำตะไคร้ | () น้ำใบบัวบก | () น้ำมะนาว |
| () น้ำดอกคำฝอย | () น้ำเห็ดหลินจือ | () น้ำกระชายดำ |
| () น้ำลูกยอ | () น้ำจับเลี้ยง | () โสม |
| () น้ำสมุนไพร อื่นๆ | ระบุ _____ | |

2. ท่านดื่มเครื่องต้มสมุนไพรบ่อยเพียงใด

- () ดื่มประจำ หรือบ่อยมาก (มากกว่า 3 ครั้งต่อสัปดาห์)
 () ดื่มบ่อยปานกลาง (1-3 ครั้งต่อสัปดาห์)
 () ดื่มน้อย นานๆ ครั้ง (น้อยกว่า 1 ครั้งต่อสัปดาห์)

3. ท่านซื้อเครื่องต้มสมุนไพรดังกล่าวจากที่ใดบ่อยที่สุด (กรุณาเลือกตอบข้อเดียว)

- | | |
|-----------------------------------|---------------------------------|
| () ห้างสรรพสินค้า/ ซูเปอร์มาเก็ต | () ร้านค้า ร้านสะดวกซื้อ |
| () ตลาดสด | () ร้านสุขภาพ ร้านเจ/มังสวิรัต |
| () งานแสดงสินค้า | () ร้านขายยา ร้านเครื่องยาจีน |
| () ไม่ได้ซื้อ ปลูกและทำเอง | () อื่นๆ ระบุ _____ |

4. วัตถุประสงค์ส่วนใหญ่ในการดื่มเครื่องต้มสมุนไพรของท่าน คือ (กรุณาเลือกตอบข้อเดียว)

- () เพื่อบำรุงสุขภาพ ให้ร่างกายแข็งแรง
 () เพื่อรักษาโรคต่างๆ
 () เพื่อเป็นเครื่องดื่มทั่วไป แก้อาการ หอบกลืนรส หรือวัตถุประสงค์อื่นๆ

5. ช่วงเวลาที่ท่านชอบดื่มเครื่องต้มสมุนไพร (ตอบได้มากกว่า 1 ข้อ)

- | | | |
|---------------------|---------------|---------------|
| () เช้า | () เที่ยง | () บ่าย |
| () กลางคืน/ก่อนนอน | () ก่อนอาหาร | () หลังอาหาร |

คำชี้แจง: กรุณาตอบคำถามต่อไปนี้ โดยขีดเครื่องหมาย ✓ ในช่องที่ตรงกับ
ความคิดเห็นของท่าน

	3. เห็นด้วย	2. ไม่แน่ใจ	1. ไม่เห็นด้วย
6. เครื่องดื่มสมุนไพร หมายถึงเครื่องดื่ม ที่มีส่วนผสมของพืชสมุนไพรเพียงอย่างเดียว			
7. เครื่องดื่มสมุนไพร อาจมีสมุนไพรมากกว่า หนึ่งอย่างผสมรวมกันอยู่			
8. น้ำส้ม น้ำมะนาว หรือน้ำผลไม้อื่นๆ เป็นเครื่องดื่มสมุนไพร			
9. น้ำแครอท น้ำคั้นช่าย หรือน้ำผักอื่นๆ เป็นเครื่องดื่มสมุนไพร			
10. เครื่องดื่มสมุนไพร จะต้องมีส่วนออกฤทธิ์ที่มีประโยชน์ ต่อร่างกาย หรือรักษาโรค			
11. เครื่องดื่มสมุนไพร มีแต่ประโยชน์ ไม่มีโทษต่อร่างกาย ได้			
12. เครื่องดื่มสมุนไพร ควรมีความปลอดภัยในการดื่ม			
13. ก่อนดื่มเครื่องดื่มสมุนไพรท่านอ่านฉลากแล้วจึงดื่ม			
14. ท่านเชื่อว่าสมุนไพรบางชนิด เช่นชาเขียว ดอกคำฝอย สามารถลดไขมันในเลือด โคเลสเตอรอล ได้			
15. ท่านคิดว่าการลดไขมันในเลือดได้ขึ้นอยู่กับอาหารการกิน			
16. ท่านคิดว่าการลดไขมันในเลือดได้ขึ้นอยู่กับ การ ออกกำลังกาย			
17. ท่านเชื่อว่าสมุนไพรมีสรรพคุณตามที่คนโบราณบอกต่อมา			

คำชี้แจง: กรุณาพิจารณาปัจจัยต่อไปนี้ที่ท่านคิดว่ามี**ความสำคัญ** เมื่อท่านจะ **บริโภค**
เครื่องตีผสมปูนไฟร โดยให้ระดับความสำคัญแล้วขีดเครื่องหมาย ✓ ในช่องที่ตรงกับคะแนน
ความสำคัญตามความคิดเห็นของท่าน มากที่สุด

	7. สำคัญ มากที่สุด	6. สำคัญ มาก	5. สำคัญ	4. เฉย ๆ	3. ไม่สำคัญ	2. ไม่สำคัญ มาก	1. ไม่สำคัญ มากที่สุด
18. ชนิด ประเภทของผสมปูนไฟร							
19. สรรพคุณ คุณสมบัติของผสมปูนไฟร							
20. ความสะอาด ปลอดภัยของ เครื่องตีผสมปูนไฟร							
21. ผลข้างเคียง หรือโทษของ เครื่องตีผสมปูนไฟร							
22. สี ของเครื่องตีผสมปูนไฟร							
23. กลิ่นของเครื่องตีผสมปูนไฟร							
24. รสชาติของเครื่องตีผสมปูนไฟร							
25. รสหวานของเครื่องตีผสมปูนไฟร							
26. ความขื่นเหน็ดของเครื่องตี ผสมปูนไฟร							
27. ความเป็นธรรมชาติ ไม่ปรุงแต่ง ใดๆ ของ เครื่องตีผสมปูนไฟร							
28. ปริมาณการตีเครื่องตีผสมปูนไฟร							
29. ระยะเวลา ความต่อเนื่องในการตี เครื่องตีผสมปูนไฟร							
30. ภาชนะบรรจุเครื่องตีผสมปูนไฟร							
31. ฉลาก หรือข้อมูล ของเครื่องตี ผสมปูนไฟร							
32. ราคาของเครื่องตีผสมปูนไฟร							
33. การโฆษณาเครื่องตีผสมปูนไฟร							
34. ความสะดวกในการบริโภคเครื่องตี ผสมปูนไฟร							
35. ความเชื่อถือ สืบต่อกันมาแต่ บรรพบุรุษ							
36. คำแนะนำจาก หมอแผนโบราณ							
37. คำแนะนำจากแพทย์แผนปัจจุบัน							

ส่วนที่ 2 ข้อมูลด้านการพัฒนาผลิตภัณฑ์เครื่องตีผสมปูนไฟรตัวอย่าง

คำแนะนำ: กรุณาทดสอบชิมผลิตภัณฑ์เครื่องตีผสมปูนไฟรตัวอย่างกรุณาให้ **คะแนนความชอบ** ในคุณลักษณะต่างๆ โดยทำเครื่องหมาย ✓ ลงในช่องคะแนนที่ตรงกับความรู้สึกของท่านมากที่สุด จากนั้นกรุณาแสดงความคิดเห็นเกี่ยวกับทิศทางการปรับปรุงพัฒนาผลิตภัณฑ์

ตัวอย่าง	ระดับความชอบต่อผลิตภัณฑ์								
	1.ไม่ชอบมากที่สุด	2.ไม่ชอบมาก	3.ไม่ชอบปานกลาง	4.ไม่ชอบเล็กน้อย	5.เฉยๆ	6.ชอบเล็กน้อย	7.ชอบปานกลาง	8.ชอบมาก	9.ชอบมากที่สุด
38.สี									
39.กลิ่น									
40.รสชาติ									
42. ความอร่อย/ ความชอบรวม									

ตัวอย่าง	ทิศทางการปรับปรุงตามความต้องการของท่าน				
	ปรับให้ลดลงมาก	ปรับให้ลดลงเล็กน้อย	ไม่ต้องปรับปรุง	ปรับให้เพิ่มขึ้นเล็กน้อย	ปรับให้เพิ่มขึ้นมาก
43.สี					
44.กลิ่นผสมปูนไฟร					
45.กลิ่นเหม็นเขียว					
46.รสขม					
47.รสหวาน					
48.รสเปรี้ยว					
49.ความฝาดเผื่อน					

50. ท่านยอมรับเครื่องตีผสมปูนไฟรตัวอย่างหรือไม่

() ยอมรับ () ไม่ยอมรับ

เหตุผล _____

51. จากเครื่องต้มสมุนไพรตัวอย่าง ท่านคิดว่าควรมีการเพิ่มกลิ่นรสอื่น หรือไม่ อย่างไร
- () ไม่ควรเพิ่มกลิ่นรสอื่น
- () ควรเพิ่มกลิ่นรสอื่นๆ กรุณาบอกต่อ
- กลิ่นรสที่ควรเพิ่ม คือ () มะลิ () เก๊กฮวย
- () น้ำผึ้ง () มะนาว
- () อื่นๆ ระบุ _____
52. ท่านชอบดื่มเครื่องดื่มสมุนไพร ลักษณะใด
- () เครื่องดื่มร้อน () เครื่องดื่มอุณหภูมิปกติ () เครื่องดื่มเย็น
53. ปริมาณเครื่องดื่มที่เหมาะสมในการดื่มต่อครั้งของท่าน
- () 100 มิลลิลิตร () 120 มิลลิลิตร () 150 มิลลิลิตร
- () 200 มิลลิลิตร () 250 มิลลิลิตร () 500 มิลลิลิตร
- () 750 มิลลิลิตร () 1 ลิตร () มากกว่า 1 ลิตร
54. เครื่องดื่มสมุนไพร รูปแบบใดที่ท่านชอบมากที่สุด
- (เลือกรูปแบบที่ชอบเพียงข้อเดียวก่อน แล้วจึงเลือกภาชนะบรรจุที่เหมาะสมของรูปแบบนั้น
-)
- รูปแบบธรรมชาติ เป็นใบแห้ง นำมาต้ม
- ภาชนะบรรจุ ถุงพลาสติก ถุงอลูมิเนียมฟอยล์
- กล่องกระดาษ ขวดพลาสติก
- รูปแบบผงบรรจุซอง ชงกับน้ำร้อน เช่น ชาซอง ชาฝรั่ง
- ภาชนะบรรจุ ถุงพลาสติก ถุงอลูมิเนียมฟอยล์
- กล่องกระดาษ ขวดพลาสติก
- รูปแบบผงสำเร็จรูปชงละลาย
- ภาชนะบรรจุ ถุงพลาสติก ถุงอลูมิเนียมฟอยล์
- กล่องกระดาษ ขวดพลาสติก
- รูปแบบพร้อมดื่ม
- ภาชนะบรรจุ ขวดพลาสติก ขวดแก้ว
- กระป๋อง กล่อง ยูเอชที

ส่วนที่ 3 ข้อมูลทั่วไปของผู้ตอบแบบสอบถาม

55. เพศ () ชาย () หญิง
56. อายุ
 () 35 – 45 ปี () 46 – 55 ปี
 () 56 – 65 ปี () มากกว่า 65 ปี
57. การศึกษา
 () ต่ำกว่าหรือเทียบเท่าประถมศึกษา () มัธยมศึกษา หรือเทียบเท่า
 () อนุปริญญา หรือเทียบเท่า () ปริญญาตรี
 () สูงกว่าปริญญาตรี
58. อาชีพ
 () นิสิต นักศึกษา () รับราชการ / รัฐวิสาหกิจ () ธุรกิจ/พนักงานบริษัทเอกชน
 () เจ้าของกิจการ / อาชีพอิสระ () รับจ้างทั่วไป () แม่บ้าน
 () เกษียณอายุ () ข้าราชการบำนาญ () ไม่ได้ทำงาน
59. รายได้ของท่านต่อเดือน
 () ไม่มีรายได้ () น้อยกว่า 5,000 บาท
 () 5,001 - 10,000 บาท () 10,001 - 15,000 บาท
 () 15,001 - 20,000 บาท () มากกว่า 20,000 บาท

.....

เมื่อมีการพัฒนาผลิตภัณฑ์เครื่องดื่มสมุนไพรนี้เสร็จแล้ว ท่านสนใจที่จะมาทดสอบต่อหรือไม่

ไม่สนใจ

สนใจ ถ้าสนใจกรุณาให้ ชื่อ ที่อยู่

ชื่อ.....

ที่อยู่.....

.....

โทรศัพท์.....

กรณารับของที่ระลึก เพื่อแทนคำขอบคุณในความร่วมมือ

Questionnaire for Consumer Survey

Topic: The consumer survey of the development of herbal beverage

Part 1: The attitude and behavior of herbal beverage consumption data

Suggestion: Please answer the questions and make ✓ in the box which is directly to your opinions.

1. The favorite herbal beverages (chose 3 items)

Please rank by 1= most liking and 3= least liking

- | | | |
|--|---|--|
| <input type="checkbox"/> Green tea/ Japanese green tea | <input type="checkbox"/> Chinese tea | <input type="checkbox"/> Ginger drink |
| <input type="checkbox"/> Chrysanthemum drink | <input type="checkbox"/> Roselle drink | <input type="checkbox"/> Basil fruit drink |
| <input type="checkbox"/> Lemon grass drink | <input type="checkbox"/> Bourbok drink | <input type="checkbox"/> Lemon juice |
| <input type="checkbox"/> Safflower drink | <input type="checkbox"/> Mushroom drink | <input type="checkbox"/> Ginseng drink |
| <input type="checkbox"/> Other herbal beverages: _____ | | |

2. Frequency of herbal beverage drinking

- Always drinking (more than 3 times a week)
- Often drinking (1-3 times a week)
- Seldom drinking (less than 1 time a week)

3. Place of buying herbal beverage (chose only one)

- | | |
|--|---|
| <input type="checkbox"/> Department store / Super market | <input type="checkbox"/> Convenient store |
| <input type="checkbox"/> Fresh market | <input type="checkbox"/> Healthy shop, Vegetarian shop |
| <input type="checkbox"/> Food fair | <input type="checkbox"/> Drug store, Chinese drug store |
| <input type="checkbox"/> Growing by myself | <input type="checkbox"/> Other: _____ |

4. The purpose of herbal beverage drinking (chose only one)

- Health benefit
- Curing the disease
- Normal beverage, thirsty, flavor etc.

5. Time to herbal beverage drinking (chose more than one)

- | | | |
|---|--------------------------------------|-------------------------------------|
| <input type="checkbox"/> Morning | <input type="checkbox"/> Noon | <input type="checkbox"/> Afternoon |
| <input type="checkbox"/> Evening, Night | <input type="checkbox"/> Before meal | <input type="checkbox"/> After meal |

Suggestion: Please put ✓ in the box which is directly to your opinions.

	3. Agree	2. Neither agree nor disagree	1. Disagree
6. Herbal beverage is the beverage that consists of single herb.			
7. Herbal beverage is the beverage that consists of mixed herbs.			
8. Orange juice, lemon juice and fruit juice are herbal beverage.			
9. Carrot juice, celery juice and vegetable juice are herbal beverage.			
10. Herbal beverage provides health benefit or cures the disease.			
11. Herbal beverage provides only benefit, no bad effect.			
12. Herbal beverage should save for drinking.			
13. I read the label before drinking the herbal beverage.			
14. I believe in some herb, such as green tea or safflower, which could reduce cholesterol level.			
15. I think that lowering cholesterol level depend on food intake.			
16. I think that lowering cholesterol level depend on the exercise.			
17. I believe the ancient in the property of herb.			

Suggestion: Please put ✓ in the box which is directly to your opinions by important rating when you consume the herbal beverage.

	7. The most important	6. Very important	5. Important	4. Neither important nor unimportant	3. Unimportant	2. Very unimportant	1. The most unimportant
18. Type of herb							
19. Properties of herb							
20. Safety of herb							
21. Side effect or bad effect of herb							
22. Color of herbal beverage							
23. Aroma of herbal beverage							
24. Taste of herbal beverage							
25. Sweetness of herbal beverage							
26. Viscosity of herbal beverage							
27. Natural flavor of herb beverage							
28. Quantity of herbal beverage drinking							
29. Time of herbal beverage drinking							
30. Package of herbal beverage							
31. Label of herbal beverage							
32. Price of herbal beverage							
33. Advertising of herbal beverage							
34. Convenient of consumption							
35. The ancient belief							
36. The ancient doctor's suggestion							
37. The medicine doctor's suggestion							

Part 2: The development data of herbal beverage sample

Suggestion: Please test the herbal beverage sample and weight the liking score by put ✓ in the box which is directly to your opinion. Then, make your opinion for develop this product.

Attributes	Liking scores								
	1. Dislike extremely	2. Dislike very much	3. Dislike moderately	4. Dislike Slightly	5. Neither like nor dislike	6. Like slightly	7. Like moderately	8. Like very much	9. Like extremely
38. Color									
39. Flavor									
40. Taste									
42. Overall liking									

Attributes	Much too much	Somewhat too much	Just right	Somewhtat too little	Much too little
43. Color					
44. Herbal Aroma					
45. Greeny odor					
46. Bitterness					
47. Sweetness					
48. Sourness					
49. Astringency					

50. Do you accept this product?

() Accept

() Reject

51. Do you want to add other flavor in this product?

No Yes.

If yes, you wants to add; Jasmine Chrysanthemum

Honey Lemon

Other: _____

52. What type of l beverage do you like?

Hot beverage Normally beverage Cool beverage

53. The volume of herbal beverage that you want to drink.

100 ml. 120 ml. 150 ml.

200 ml 250 ml 500 ml

750 ml 1 L. > 1 L.

54. What type of herbal beverage do you want?

Dried leaf, brewing

Powder in tea bag

Instant powder

Ready to drink

Part 3: Demographic data

55. Sex Male Female

56. Age

35-45 years old 46-55 years old

56-65 years old over 65 years old

57. Education

Primary school High school Vocational degree

Bachelor degree Higher degree

58. Occupation

Student Government officer Business

House wife Retirement Unemployed

59. Income

None less than 5,000 baht

5,001-10,000 bath 10,001-15,000 baht

15,001-20,000 bath more than 20,000 baht

Thank you for your corporation

Please take the souvenir

APPENDIX 5

Panel Selection and Training for Descriptive Analysis

Panel Selection and Training for Descriptive Analysis

Selection of the Panelist. (ASTM, 1981; Meilgaard, Civille and Carr, 1999) For a panel of 13 from 30 candidates will screen by Pre-screen questionnaire and scaling exercises which were 80% passed.

Matching and Description test : Taste and Aroma matching and description test were used selective panelists. Describe the sensory impression of products; fragrance, flavor, odorant. Candidates should be able to describe 80% of stimuli (Meilgaard et al., 1999) and should at least attempt to describe the remainder with less specific terms.

Detection: 12 Triangle discrimination tests, each replicated twice, use for selective subjects who achieve 66% correct replies, out of the total of 24 (ASTM, 1981).

Training for Descriptive Testing.

Terminology Development. Panelists individually examine the sample, generate descriptive terms and follow by discussion (Cairncross and Sjostrom, 1997; Lawless and Heymann, 1998; Grosso and Resurreccion, 2002). The panels discussed the attributes and agree on common descriptors. Redundant terms will be subsequently grouped and named an appropriate term resulting for reduction. Thirteen attributes were developed and defined (Table 7). The definitions of terms will be proposed. Reference standards will be decided by panelist consensus.

Introduction to Descriptive Scale. The graphic scale was used to evaluate the intensity of each of the sensory attributes is an unstructured horizontal line 15 cm.(6 inch) in length usually anchored 12.5 cm (1/2 inch) from each end by a pair of terms which describe or limit the attribute (ASTM, 1992). Reference rating and control Jiaogulan tea were decided by panelist consensus. Some references were obtained from the literature. Reference standards played an important role in sensory analysis

(Wolfe, 1979) by helping panelists relate to a specific perception (Stone and Sidel, 1993)

Training. Panelists were trained to evaluate the sensory characteristics of Jiaogulan tea and descriptive scale using reference standard and worm up sample to increase reliability of ratings (Plemmons and Resurreccion, 1998)

APPENDIX 6

Descriptive Ballot

Descriptive Ballots
JIAOGULAN TEA

Name _____ Date _____ Code _____

Place a vertical mark through the line scale to indicate the intensity of each attribute

(the scale is from 0 to 150) Please stir 3 times with a spoon provided.

Appearance

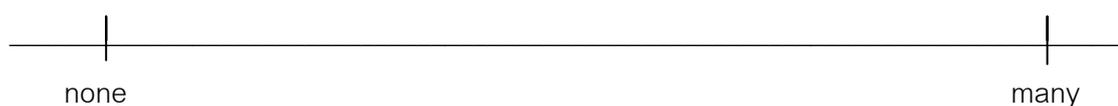
Green yellow color = Light yellow to drak green

Reference: Tratarzine = 45, Bromocresol green = 120, Jiaogulan tea control = 70



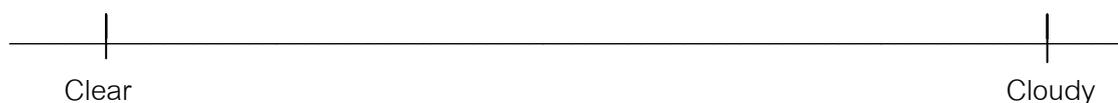
Sedimentation = The degree of sedimentary of sample

Reference: 0.01% tea powder = 75, 0.05% tea powder = 130, Jiaogulan tea control = 23



Clearness = The degree of clarity of sample

Reference: Water = 0, Corn starch solution = 150, Jiaogulan tea control = 47



Aroma

Dried leaf smell = Aromatic associated with dried grass or rice leaf or bamboo leaf

Reference : Dried Jiaogulan = 30, Jiaogulan tea control =32



Green tea smell = Aromatic associated with green tea

Reference: Japanese green tea = 25 , Jiaogulan tea control = 40



Jiaogulan smell = Aromatic associated with Jiaogulan

Reference: Jiaogulan tea control = 64



Taste

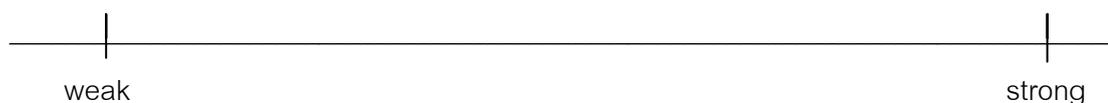
Sweet = The taste on the tongue associated with aqueous solution of sugar

Reference: Standard solution of sucrose = 20, 50, 10, 150, Jiaogulan tea control = 32



Bitter = The taste on the tongue associated with aqueous solution of caffeine

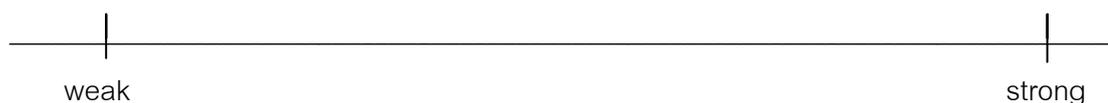
Reference: Standard solution of caffeine = 20, 50, Jiaogulan tea control = 51



Flavor

Green tea flavor = Flavor associated with green tea

Reference : Japanese green tea = 25 , Jiaogulan tea control = 33



Jiaogulan flavor = Flavor associated with Jiaogulan

Reference : Jiaogulan tea control = 62



Feeling factor

Astringency = The shrinking of the tongue surface caused by alum

Reference : 0.07% Alum = 10, 0.3% Alum = 30, 1 hr. tea bag = 95, Jiaogulan tea control=42



Aftertaste

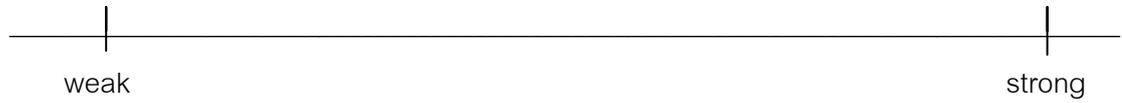
Sweet = The sweet taste after product is swallowed

Reference: Standard solution of sucrose = 5, 25, 45, 85, Jiaogulan tea control = 23



Bitter = The bitter taste after product is swallowed

Reference: Standard solution of caffeine = 27, 80, Jiaogulan tea control = 60



Astringent = The shrinking of feeling after product is swallowed

Reference: 0.07%Alum = 5, 0.3%Alum = 80, 1 hr. tea bag = 110, Jiaogulan tea control = 59



Comment

: _____

Thank you for cooperation

APPENDIX 7

Questionnaire for Consumer Acceptance Test

Questionnaire for Consumer Acceptance test (in Thai)

แบบสอบถาม

เรื่อง การทดสอบการยอมรับของผู้บริโภคต่อผลิตภัณฑ์ชาเขียวกู่หลาน

เรียน ท่านผู้ตอบแบบสอบถาม

แบบสอบถามนี้เป็นการทดสอบการยอมรับของผู้บริโภคที่มีต่อ ผลิตภัณฑ์ชาเขียวกู่หลาน เพื่อใช้ ประกอบการเรียบเรียงวิทยานิพนธ์ของ นางนิรมล อุตมอ่าง นักศึกษาระดับปริญญาเอก สาขาวิชา พัฒนาผลิตภัณฑ์อุตสาหกรรมเกษตร มหาวิทยาลัยเกษตรศาสตร์ ภายใต้การดูแลของ รศ.ดร.เพ็ญขวัญ ชมปรีดา โดยได้รับทุนสนับสนุนงานวิจัยจากมูลนิธิโครงการหลวง ซึ่งผู้วิจัยใคร่ขอ ความกรุณาและ ความร่วมมือจากท่านในการตอบแบบสอบถามให้สมบูรณ์

โดยแบบสอบถามแบ่งเป็น 3 ส่วน ดังนี้

ส่วนที่ 1 ข้อมูลทั่วไปของผู้ตอบแบบสอบถาม

ส่วนที่ 2 ข้อมูลด้านการยอมรับผลิตภัณฑ์ โดยมีการทดสอบชิม ตัวอย่าง

ส่วนที่ 3 ข้อมูลรายละเอียดเกี่ยวกับตัวผลิตภัณฑ์

(กรุณาทำที่ละหน้า อย่าเปิดอ่าน ล่วงหน้า)

ทั้งนี้ข้อมูลทั้งหมดที่ท่านตอบมจะเป็นประโยชน์อย่างยิ่งสำหรับงานวิจัยดังกล่าว ขอขอบคุณท่านที่เสียสละเวลาในการตอบแบบสอบถามในครั้งนี้เป็นอย่างสูง

ผู้วิจัย

แบบสอบถาม

เรื่อง การทดสอบการยอมรับของผู้บริโภคต่อผลิตภัณฑ์ชาเขียวกู่หลาน

เฉพาะเจ้าหน้าที่

ส่วนที่ 2 ข้อมูลทั่วไปของผู้ตอบแบบสอบถาม

9. ความชอบคุณภาพโดยรวม

<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>				
1.ไม่ชอบ อย่างยิ่ง	2.ไม่ชอบ มาก	3.ไม่ชอบ ปานกลาง	4.ไม่ชอบ เล็กน้อย	5.บอกไม่ได้ว่า ชอบหรือไม่ชอบ	6.ชอบ เล็กน้อย	7.ชอบ ปานกลาง	8.ชอบ มาก	9.ชอบ อย่างยิ่ง

10. สี

<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>				
1.ไม่ชอบ อย่างยิ่ง	2.ไม่ชอบ มาก	3.ไม่ชอบ ปานกลาง	4.ไม่ชอบ เล็กน้อย	5.บอกไม่ได้ว่า ชอบหรือไม่ชอบ	6.ชอบ เล็กน้อย	7.ชอบ ปานกลาง	8.ชอบ มาก	9.ชอบ อย่างยิ่ง

11. กลิ่น

<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>				
1.ไม่ชอบ อย่างยิ่ง	2.ไม่ชอบ มาก	3.ไม่ชอบ ปานกลาง	4.ไม่ชอบ เล็กน้อย	5.บอกไม่ได้ว่า ชอบหรือไม่ชอบ	6.ชอบ เล็กน้อย	7.ชอบ ปานกลาง	8.ชอบ มาก	9.ชอบ อย่างยิ่ง

12. รสชาติรวม

<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>				
1.ไม่ชอบ อย่างยิ่ง	2.ไม่ชอบ มาก	3.ไม่ชอบ ปานกลาง	4.ไม่ชอบ เล็กน้อย	5.บอกไม่ได้ว่า ชอบหรือไม่ชอบ	6.ชอบ เล็กน้อย	7.ชอบ ปานกลาง	8.ชอบ มาก	9.ชอบ อย่างยิ่ง

13. รสหวาน

<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>				
1.ไม่ชอบ อย่างยิ่ง	2.ไม่ชอบ มาก	3.ไม่ชอบ ปานกลาง	4.ไม่ชอบ เล็กน้อย	5.บอกไม่ได้ว่า ชอบหรือไม่ชอบ	6.ชอบ เล็กน้อย	7.ชอบ ปานกลาง	8.ชอบ มาก	9.ชอบ อย่างยิ่ง

14. รสขม

<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>				
1.ไม่ชอบ อย่างยิ่ง	2.ไม่ชอบ มาก	3.ไม่ชอบ ปานกลาง	4.ไม่ชอบ เล็กน้อย	5.บอกไม่ได้ว่า ชอบหรือไม่ชอบ	6.ชอบ เล็กน้อย	7.ชอบ ปานกลาง	8.ชอบ มาก	9.ชอบ อย่างยิ่ง

15. ความรู้สึกหลังกลืน

<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>				
1.ไม่ชอบ อย่างยิ่ง	2.ไม่ชอบ มาก	3.ไม่ชอบ ปานกลาง	4.ไม่ชอบ เล็กน้อย	5.บอกไม่ได้ว่า ชอบหรือไม่ชอบ	6.ชอบ เล็กน้อย	7.ชอบ ปานกลาง	8.ชอบ มาก	9.ชอบ อย่างยิ่ง

16. ท่านยอมรับผลิตภัณฑ์ชาเขียวกู่หลานตัวอย่างหรือไม่ () ยอมรับ () ไม่ยอมรับ

17. ถ้ามีผลิตภัณฑ์ชาเขียวกู่หลานนี้จำหน่ายในตลาด ท่านเต็มใจจะซื้อผลิตภัณฑ์นี้หรือไม่

() ซื้อ () ไม่ซื้อ

ส่วนที่ 3 ข้อมูลด้านรายละเอียดเกี่ยวกับผลิตภัณฑ์ชาเขียวกู่หลาน จากงานวิจัยนี้

ชาเขียวกู่หลาน

Part 1: Demographic data

1. Sex Male Female

2. Age

35-45 years old 46-55 years old

56-65 years old over 65 years old

3. Education

Primary school High school Vocational degree

Bachelor degree Higher degree

4. Occupation

Student Government officer Business

House wife Retirement Unemployed

5. Income

None less than 5,000 baht

5,001-10,000 baht 10,001-15,000 baht

15,001-20,000 baht 20,001-25,000 baht

25,001-30,000 baht more than 30,000 baht

Part 2: Acceptance test of Jiaogulan tea

Suggestion: Please test the Jiaogulan tea sample and weight your liking score by put

✓ in the box which is directly to your opinion.

6. Overall liking

<input type="checkbox"/> 1. Dislike extremely	<input type="checkbox"/> 2. Dislike Very much	<input type="checkbox"/> 3. Dislike moderately	<input type="checkbox"/> 4. Dislike slightly	<input type="checkbox"/> 5. Neither like nor dislike	<input type="checkbox"/> 6. Like Slightly	<input type="checkbox"/> 7. Like moderately	<input type="checkbox"/> 8. Like very much	<input type="checkbox"/> 9. Like extremely
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7. Color

<input type="checkbox"/> 1. Dislike extremely	<input type="checkbox"/> 2. Dislike Very much	<input type="checkbox"/> 3. Dislike moderately	<input type="checkbox"/> 4. Dislike slightly	<input type="checkbox"/> 5. Neither like nor dislike	<input type="checkbox"/> 6. Like Slightly	<input type="checkbox"/> 7. Like moderately	<input type="checkbox"/> 8. Like very much	<input type="checkbox"/> 9. Like extremely
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8. Aroma

<input type="checkbox"/> 1. Dislike extremely	<input type="checkbox"/> 2. Dislike Very much	<input type="checkbox"/> 3. Dislike moderately	<input type="checkbox"/> 4. Dislike slightly	<input type="checkbox"/> 5. Neither like nor dislike	<input type="checkbox"/> 6. Like Slightly	<input type="checkbox"/> 7. Like moderately	<input type="checkbox"/> 8. Like very much	<input type="checkbox"/> 9. Like extremely
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9. Overall flavor

<input type="checkbox"/> 1. Dislike extremely	<input type="checkbox"/> 2. Dislike Very much	<input type="checkbox"/> 3. Dislike moderately	<input type="checkbox"/> 4. Dislike slightly	<input type="checkbox"/> 5. Neither like nor dislike	<input type="checkbox"/> 6. Like Slightly	<input type="checkbox"/> 7. Like moderately	<input type="checkbox"/> 8. Like very much	<input type="checkbox"/> 9. Like extremely
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10. Sweetness

<input type="checkbox"/> 1. Dislike extremely	<input type="checkbox"/> 2. Dislike Very much	<input type="checkbox"/> 3. Dislike moderately	<input type="checkbox"/> 4. Dislike slightly	<input type="checkbox"/> 5. Neither like nor dislike	<input type="checkbox"/> 6. Like Slightly	<input type="checkbox"/> 7. Like moderately	<input type="checkbox"/> 8. Like very much	<input type="checkbox"/> 9. Like extremely
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11. Bitterness

<input type="checkbox"/> 1. Dislike extremely	<input type="checkbox"/> 2. Dislike Very much	<input type="checkbox"/> 3. Dislike moderately	<input type="checkbox"/> 4. Dislike slightly	<input type="checkbox"/> 5. Neither like nor dislike	<input type="checkbox"/> 6. Like Slightly	<input type="checkbox"/> 7. Like moderately	<input type="checkbox"/> 8. Like very much	<input type="checkbox"/> 9. Like extremely
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12. Aftertaste

<input type="checkbox"/> 1. Dislike extremely	<input type="checkbox"/> 2. Dislike Very much	<input type="checkbox"/> 3. Dislike moderately	<input type="checkbox"/> 4. Dislike slightly	<input type="checkbox"/> 5. Neither like nor dislike	<input type="checkbox"/> 6. Like Slightly	<input type="checkbox"/> 7. Like moderately	<input type="checkbox"/> 8. Like very much	<input type="checkbox"/> 9. Like extremely
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13. Do you accept this product?

() Yes () No

14. Do you want to buy this product?

() Yes () No

Part 3: The research information of Jiaogulan tea

<p>Jiaogulan Tea</p>

Jiaogulan is a Chinese medicine herb which is health benefit. Now it can grow in Thailand, such as Chiangmai, Chiangrai and Mae Hong Song.

Jiaogulan consists of active compounds, saponins and antioxidants. The research results indicated that saponin in Jiaogulan is the same compound as Ginseng, ginsenoside Rb1 and Rg1.

In vivo study, Jiaogulan tea can reduce triglyceride and lower cholesterol level.

After reading this information, please answer the questions.

15. Do you accept this product?

Yes No

16. Do you want to buy this product?

Yes No

17. The optimum price of Jiaogulan tea per bag

5 bath 6 bath 7 bath

Thank you for your corporation

Please take the souvenir

CURRIVULUM VITAE

NAME : Mrs. Niramon Utama-ang

BIRTH DATE : May 11, 1966.

EDUCATION	: <u>YEAR</u>	<u>INSTITUE</u>	<u>DEGREE</u>
	1987	Chiangmai University	B.S. (Food Science and Technology),
	1993	Kasetsart University	M.S. (Agro-Industrial Product Development)

WORKPLACE : Department of Product Development Technology,
Faculty of Agro-Industry, Chiangmai University

SCHOLARSHIP : The Royal Project Foundation, Thailand
The Agro-Industrial Consortium Program, The Ministry of
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