การพัฒนาผลิตภัณฑ์เครื่องดื่มสมุนไพรรวมสเตอริไลส์

นายบดินทร์ เตชะรัตนไกร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเทคโนโลยีทางอาหาร ภาควิชาเทคโนโลยีทางอาหาร
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2551

DEVELOPMENT OF STERILIZED MIXED HERBAL DRINK PRODUCT

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Food Technology
Department of Food Technology
Faculty of Science
Chulalongkorn University
Academic Year 2008

Thesis Title	DEVELOPMENT OF STERILIZED MIXED HERBAL DRINK PRODUCT
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บดินทร์ เตชะรัตนไกร : การพัฒนาผลิตภัณฑ์เครื่องดื่มสมุนไพรรวมสเตอริไลส์. (DEVELOPMENT OF STERILIZED MIXED HERBAL DRINK PRODUCT) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : รศ.ดร.สายวรุฬ ชัยวานิชศิริ, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม : รศ.ดร.กัลยา เลาหสงคราม, 94 หน้า.

งานวิจัยนี้จึงมีวัตถุประสงค์เพื่อพัฒนาสูตรและกระบวนการสเตอริไลส์เครื่องดื่มสมุนไพรรวมจากสมุนไพร 4 ชนิด ได้แก่ หญ้าคา หญ้าปักกิ่ง หญ้าลิ้นงู และหญ้าหนวดแมว โดยเริ่มจากการวิเคราะห์องค์ประกอบทางเคมีและปริมาณโปแตสเซียมของวัตถุดิบ จากนั้นศึกษาสภาวะที่เหมาะสมในการเตรียมน้ำสกัด โดยใช้อัตราส่วนของสมุนไพรแห้งต่อน้ำเป็น 1:49 แปรอุณหภูมิและเวลาในการสกัดเป็น 70, 80 และ 90 องศาเซลเซียส และ 30, 45 และ 60 นาที ตามลำดับ จากนั้นพัฒนาเครื่องดื่มสมุนไพรรวมเพื่อให้ได้สูตรที่มีสัดส่วนของน้ำสมุนไพรแต่ละชนิดและปริมาณน้ำตาลที่ได้รับการยอมรับจากผู้บริโภคสูงสุด และศึกษาสภาวะที่เหมาะสมในการสเตอริไลส์ผลิตภัณฑ์เครื่องดื่มสมุนไพรรวมบรรจุขวด PET โดยแปรอุณหภูมิการฆ่าเชื้อ 130, 135 และ 140 องศาเซลเซียส และเวลาเพื่อให้ได้ค่า F_0 เท่ากับ 3, 4 และ 5 นาที วิเคราะห์สมบัติทางกายภาพ เคมี จุลชีววิทยา และการยอมรับของผู้บริโภคของผลิตภัณฑ์หลังผ่านกระบวนการสเตอริไลส์ ขั้นตอนสุดท้าย เก็บผลิตภัณฑ์ที่ได้ที่อุณหภูมิ 30 องศาเซลเซียส เป็นเวลา 4 เดือน และที่ 45 และ 55 องศาเซลเซียสเป็นเวลา 2 เดือน ติดตามการเปลี่ยนแปลงสมบัติทางกายภาพ เคมี จุลชีววิทยา และการยอมรับของผู้บริโภคทุกสัปดาห์

จากการศึกษาพบว่าสมุนไพรแห้งทั้ง 4 ชนิดมีปริมาณใยอาหารสูง (24.11-60.45%) และมีปริมาณโปรตีน กับไขมันค่อนข้างต่ำ (1.16-10.96% และ 0.46-10.12% ตามลำดับ) โดยหญ้าปักกิ่งและหญ้าหนวดแมวมีปริมาณโปแตสเซียมสูง คือ 2.01 และ 1.35% ตามลำดับ เมื่อนำสมุนไพรทั้ง 4 ชนิดมาสกัด พบว่าน้ำสกัดสมุนไพรมีสีเข้มขึ้น ส่วนค่ากิจกรรมด้านอนุมูลอิสระและปริมาณวิตามินซีลดลง แต่ปริมาณสารประกอบฟีนอล และปริมาณโปแตสเซียมเพิ่มขึ้นเมื่อเวลาและอุณหภูมิในการสกัดเพิ่มขึ้น จากการใช้เทคนิค response surface พบว่าสภาวะในการสกัดที่ดีที่สุด คือ การสกัดที่อุณหภูมิ 74.6 องศาเซลเซียส เป็นเวลา 41 นาที จากการพัฒนาสูตรเครื่องดื่มสมุนไพรจากน้ำสกัดสมุนไพร พบว่าสูตรน้ำสมุนไพรที่ได้รับการยอมรับสูงสุด ประกอบด้วย น้ำหญ้าคา 14%, น้ำหญ้าปักกิ่ง 35%, น้ำหญ้าลิ้นงู 27%, น้ำหญ้าหนวดแมว 17% และน้ำตาล 7% จากการสเตอริไลส์น้ำสมุนไพรผสม พบว่าค่า F_0 ที่เพิ่มขึ้นส่งผลให้น้ำสมุนไพรมีสีเข้มขึ้น ค่ากิจกรรมด้านอนุมูลอิสระและปริมาณสารประกอบฟีนอลลดลง แต่ไม่มีผลต่อค่า pH, ปริมาณของแข็งละลายน้ำทั้งหมด และปริมาณโปแตสเซียม นอกจากนี้น้ำสมุนไพรที่ผ่านการสเตอริไลส์ทุกสภาวะตรวจไม่พบเชื้อจุลินทรีย์ และสภาวะในการสเตอริไลส์ที่ดีที่สุด คือ 135.4 องศาเซลเซียส ค่า F_0 เท่ากับ 3 นาที และจากการศึกษาการเปลี่ยนแปลงคุณภาพระหว่างเก็บ พบว่าเมื่อระยะเวลาการเก็บนานขึ้น ผลิตภัณฑ์มีสีเข้มขึ้น กิจกรรมด้านอนุมูลอิสระและปริมาณสารประกอบฟีนอลทั้งหมดลดลง แต่ความชอบโดยรวมเพิ่มสูงขึ้น แต่ไม่ส่งผลต่อค่า pH และปริมาณของแข็งละลายน้ำทั้งหมด และตรวจไม่พบเชื้อจุลินทรีย์ในตัวอย่างทั้งหมด โดยจากการคำนวณพบว่าปริมาณสารประกอบฟีนอลลดลงจนหมดหลังการเก็บน้ำสมุนไพรสเตอริไลส์เป็นเวลา 27 สัปดาห์ ที่อุณหภูมิการเก็บ 30 องศาเซลเซียส

ภาควิชา.....เทคโนโลยีทางอาหาร..... ลายมือชื่อนิสิต.....

สาขาวิชา.....เทคโนโลยีทางอาหาร..... ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ปีการศึกษา.....2551.....ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

4972602623 : MAJOR FOOD TECHNOLOGY

KEY WORD: *Imperata cylindrical* (L.) P. Beauv. / *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy / *Hedyotis corymbosa* Lamk. / *Orthosiphon aristatus* Miq. / sterilization

BODIN TECHARATANAKRAI : DEVELOPMENT OF STERILIZED MIXED HERBAL DRINK PRODUCT. THESIS PRINCIPAL ADVISOR : ASSOC. PROF. SAIWARUN CHAIWANICHSIRI, Ph.D., THESIS COADVISOR : ASSOC. PROF. KALAYA LAOHASONGKRAM, Ph.D., 94 pp.

This research aimed to develop sterilized mixed herbal drink product formula and processes from *Imperata cylindrical* (L.) P. Beauv. [*IM*], *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy [*ML*], *Hedyotis corymbosa* Lamk. [*HC*], and *Orthosiphon aristatus* Miq [*OA*]. The chemical composition and potassium content of the herbs were analyzed. The appropriate extraction condition using herb: water ratio of 1: 49, and extracting at 70 °, 80 ° and 90 °C for 30, 45 and 60 minutes were investigated. The formula of mixed herbal drink was developed to determine the proportion of each herbal infusion and sugar level. The most acceptable formula of mixed herbal drink was then sterilized at 130 °, 135 ° and 140 °C to obtain F_0 of 3, 4 and 5 minutes and filled in PET bottle then all samples were investigated. The sterilized mixed herbal drink was kept at 30 °C for 4 months and at 45 ° and 55 °C for 2 months. Changes in the physical, chemical, microbiological properties and panelists' preference of the samples were determined every week during storage.

From the study, all herbs had high amount of fiber (24.11-60.45%) and low amount of protein (1.16-10.96%) and fat (0.46-10.12%). *ML* and *OA* had the high amount of potassium (2.01% and 1.35%). Increasing extraction temperature and time caused darker product, reduction in antioxidant activity and ascorbič acid but increasing in total phenolic compounds and potassium. From response surface methodology, the most appropriate extraction condition was 74.6 °C for 41 minutes. The most acceptable formula contains 14% *IC* infusion, 35% *ML* infusion, 27% *HC* infusion, 17% *OA*, and 7% sugar. Increase F_0 in sterilization step resulted in darker product and reduction in antioxidant activity and total phenolic compounds, but did not affect pH, total soluble solid, and potassium. Moreover, no microorganism was detected in all samples. The most appropriate sterilization process was 135.4 °C with F_0 = 3 minutes. Increasing storage time and temperature caused a decrease in antioxidant activity and total phenolic compounds and increase in panelists' preference. Total phenolic compoundswere predicted to deplete absolutely in the week 27th at 30 °C.

Department.....Food Technology..... Student's signature.....

Field of study.....Food Technology..... Principal Advisor's signature.....

Academic year.....2008..... Co-advisor's signature.....

Acknowledgement

I, as the researcher, would like to express my gratitude to Associate Professor Dr. Saiwarun Chaiwanichsiri and Associate Professor Dr. Kalaya Laohasongkram as advisor and co-advisor for giving good suggestions, advice and comments. Moreover, they are driving force to complete this thesis.

I sincerely thank to Assistant Professor Dr. Rommanee Sanguandeekul as the chairman thesis committee, Assistant Professor Dr. Varapha Kongpensook and Mr. Narongchai Dharmsuriya, for giving me their precious time to be thesis committee members and comments to improve this thesis.

I am grateful to thank these people and agencies for financial and instrumental granting

- Graduate School of Chulalongkorn University for research financial grant
- Thailand Research Fund - Master Research Grant for research financial grants
- Mr. Jirawat Lohavanichbutr for giving a chance to use UHT/HTST processing system (Microthermics Bantam-DH, U.S.A.) and ultra-clean fill hood (Microthermics Clean Fill Hood, U.S.A.)

I would like to thank every professors and officers in the department for giving advice, comfort, and willpower to let me finish this research

I thank graduate, postgraduate students and concerned people for sacrificing their time to be panelists.

Finally, I would like to express my gratitude to my mother for supporting me during my Master's degree study with every mean.

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Chapter I

Introduction

Nowadays, humans are concerned about health as their lifestyle, including consuming behavior, are changed. The evidences that prove this situation are the increasing growth rate of fruit juice in the market from 15% in 2002 to 40% in 2006 (Modern Brewery Age: Battle in Beverage Market, 2006) and the 7% increase in nutritional supplements in 2007 from 2006 (Kasikorn Research Center, 2007).

In Thailand, herbal drinks are widely developed, especially in household scale. However, industries produce herbal drinks in limited variety, such as chrysanthemum, bael, ginger, etc. Thailand has 4,087 kinds of herb but only 974 species are used in practice (Somboon Kietinan, 2007). So this research aims to develop herbal product from four herbs namely *Imperata cylindrical* (L.) P. Beauv., *Murdannia loriformia*, *Hedyotis corymbosa* Lamk., and *Orthosiphon aristatus*, whose beneficial effects are reported in both traditional and modern medical techniques.

Chapter II

Literature Review

2.1 Herb and Herbal Tea

Herbs are plants which valued for their flavor, fragrance, medicinal and healthful qualities, economic and industrial uses, pesticidal properties, and coloring materials (dyes). (Bown, 2001). Herb can be used in many industries; for instance, traditional medicine, animal medicine, cosmetics, agriculture, sanitary ware, spa, packaging, mouthwash, feed and food. (Vichai Haruthaithanasan, 2007)

One of the application of herb in food industry is herbal tea or herbal infusion. From the Notice of Ministry of Public Health (2004), herbal tea is a product from any part of nonprocessed plant for consuming by boiling or infusing with water. In this notice limit 15 herbs, which are bael fruit, rosella, ginger, galangal, lemon grass, white mulberry, safflower, asiatic pennywort, pandanus, chrysanthemum, luo han gua, reishi, Indian gooseberry, jiaogulan, and jewel vine. Others are announced by Food and Drug Administration Thailand. However, herbal tea must not contain other ingredients such as sugar or honey, or else it cannot be called herbal tea.

2.2 *Imperata cylindrical* (L.) P. Beauv.

Imperata cylindrical (L.) P. Beauv., called thatch grass, wolly grass, lalang or alang-alang (Phayao Muanwongyat, 2002), is in Gramineae Family. It is a short-lived plant, which has white cylindrical, cleared jointed, smooth or little hairy underground stem. Its leaves are flat, straight and long, about 20 - 50 cm in length and 5 - 9 mm in width. This grass has inflorescences, composed white florets which have cylindrical shape that have 5-20 cm in length and 1.5-3 cm in diameter (Chaiyo Chaichantipyuth, 1982)

In traditional medicine, water extract from its root is used for diuretic, cystitis treatment, kidney maintenance, leukorrhea driving, ahpthous ulcer treatment, jaundice treatment, hypertension treatment, oedema treatment, gonorrhea treatment, and

menorrhagia treatment (Wutti Wuttidhammavech, 1997). From some experiment, water extract from its root can treat hepatitis too (Chaiyo Chaichantipyuth, 1982).

Root of Imperata cylindrical (L.) P. Beauv. is composed of 21% dry basis sugar such as sucrose, glucose, fructose (Chaiyo Chaichantipyuth, 1982). Moreover, there are many essential substances, e.g. arundoin, cylindrin and many organic acids. (Wandee Gritanapan, 1994)

Figure 2.1 *Imperata cylindrical* (L.) P. Beauv.
From: Lippincott (1997)

2.3 *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy

Murdannia loriformia (Hassk.) Rolla Rao et Kammathy is in Commelinaceae Family. It is a short-lived plant that has about 7 - 20 cm tall. Its leaves at stem are about 1 cm in

width and 10 cm or lower in length. (Worapot Suntornsuk *et al.*, 2004).

In traditional medicine, its stems and leaves can be used for throat ache treatment, and cancer treatment (Wutti Wuttidhammavech, 1997). Nowaday, this herb is used along with modern medicine for side effect reduction. There are many reported that cancer-suffered patients use this herb for self-treatment; for example, lung, gastric, and urethral cancers, leukemia, and brain tumor (Sakorn Pornprasert, Khanittha Punturee, and Usanee Vinitketkumneun, 2001). From *in vitro* study, this plant has cytotoxic compounds for human breast, colon, lung, and liver cancer cells (Weena Jiratchariyakul *et al.*, 2006). Another study shows that the extract of this grass make T-cell and B-cell proliferation decreased strongly (Khanittha Puntaree *et al.*, 2005).

Leaves and stems of *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy have glycosidic compounds such as digalactosyl diglyceride, isovotexin, syringic acid, and glycosphingolipids (Wandee Gritanapan, 1994).

Figure 2.2 *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy
From: Oauporn Chaiwan (2006)

2.4 *Hedyotis corymbosa* Lamk.

Hedyotis corymbosa Lamk. or *Oldenlandia corymbosa* Lamk. is a member of Rubiaceae. This short-lived plant is about 25 cm tall, often branched in base. Its opposite leaves are 10-35 mm in length and 2-7 mm in width. This plant has globular fruit with 2 mm in diameter (Liu and Yang, 2003).

In traditional medicine, all part of this herb can be used for malaria treatment, gastroenteritis treatment, bathing abscess, burned wound (Wutti Wuttidhammavech, 1997: 393). This herb has nine groups of substance which have high toxicity to cancer cell (Kamonwan Anan *et al.*, 2006). Moreover, this plant has potent hepatoprotective against upon paracetamol-induced hepatic damage in rats and possessed antilipid peroxidative and free radical scavenging activities (Sadasivan *et al.*, 2006).

Figure 2.3 *Hedyotis corymbosa* Lamk.
From: Davis (2002a)

2.5 *Orthosiphon aristatus* Miq.

Orthosiphon aristatus Miq., also called kidney tea plant or java tea, is a short-lived plant that about 0.3 - 1 m tall. Its single, opposite leaves have diamond shape with dark green, rough edge. Its inflorescences have light violet florets. This plant has long stamens, which are the dominant feature of this plant, like cat's whisker (Phayao Muanwongyat, 1994).

In traditional medicine, this herb can be used for diuretic, kidney disease treatment, kidney stone treatment, diabetes treatment, and hypertension treatment (Nuntavan Bunyapraphatsara, ed., 1987). This herb has potassium salt and orthosiphonin as essential substances (Laddawan Boonyaratanakornkit, 1992). There are some experiments which show that this plant helps patients who suffered from inability to urinate because of benign prostatic hypertrophy. Moreover, it forces kidney stone out of the body (Weena Jiratchariyakul, 1991).

Figure 2.4 *Orthosiphon aristatus* Miq.
From: Davis (2002b)

2.6 Sterilization

Sterilization is the unit operation in which foods are heated at a sufficient high temperature and for a sufficiently long time to destroy microbial and enzyme activity. As a result, sterilized foods have a long shelf life at ambient temperatures (Fellows, 2000: 250). However, thermal sterilization process can be beneficial or detrimental to quality and nutritional compounds (Meenakshi, 2007).

Conventional sterilization of asparagus caused the reduction of rutin from 0.45 mg/g wet weight to 0.33 mg/g wet weight and antioxidant activity lost from 2.87 μmol trolox eq/g wet weight to 2.99 μmol trolox eq/g wet weight. Moreover, the sterilization process caused darker and browner asparagus and softened the product (Sun, Tang, and Powers, 2007).

Six vegetable extracts, spinach, komatsuna, chingensai, haruna, Chinese cabbage, and white cabbage, after heating process lost phenolic compounds, anti-DPPH activity, inhibitory activity against hydroxyl radical, and anti-proliferative activity against HL-60 cell line (Roy *et al.*, 2007).

Antioxidant activity in black chokeberry juice concentrate was decreased by 1.2-5.8% after heating process under facultative anaerobic condition and by 6.0-15.8% under aerobic condition (Walkowiak-Tomczak, 2007).

Thermal treatment affects carotenoid pigment contents of Brazilian Valencia orange juice, especially violaxanthin and lutein by 38% and 20%, respectively (Gama and Sylos, 2007).

In cloudy apple juice, thermal treatment caused browning effect on the product and gave the stabiity of cloud in juice. Polyphenoloxidase and pectinesterase activity were both reduced after treatment (Krapfenbauer *et al.*, 2006).

Min, Chunli, and Ping (2004) found that chlorophyll, total selenium content, especially organic selenium, in Chinese cabbage was decreased after sterilization process.

Thermal process inhibited *Saccharomyces cerevisiae* recovery in *Parellada* grape juice. However, thermal processing did not significantly affect the amount of total fatty acids and total amino acids in juice (Garde-Cerd?n *et al.*, 2007).

Total β-carotene content of sterilized carrot juice did not greatly differ from the unheated carrot juices. Heat preservation had an additive effect on isomerization and lead to approximately 5% of increasing of 13-cis-β-carotene. The more severe sterilization condition enhanced trans-cis-isomerization and formed 9-cis-β-carotene (Marx *et al.*, 2003).

According to Zazoni and coworkers (2003), furosine content in tomato puree increased but ascorbic acid and antioxidant activity of the hydrophilic fraction decreased and color variation was observed. However, no significant variations was found in lycopene content, antioxidant activity of lipophilic fraction and sensory evaluation.

Sterilization process caused the browning effect and cooked flavor of volatile sulfur compounds or from reaction between reducing sugars and proteins, from lactone formation in milk. The loss of many vitamins, especially vitamin B_6 and B_{12}, were also found (Jordan, 1967).

2.7 Antioxidant

Free radical is one of the main reasons causing more than one hundreds disorders such as atherosclerosis, ischemia, cancer and AIDS. Free radicals can come from environmental pollutants, radiation, chemicals, toxins, physical stress or deep fried and spicy foods. These radicals will deplete immune system antioxidants, change gene expression and induce abnormal proteins. Oxidation process plays a role for producing free radicals in food, drugs and living systems (Pourmorad, Hosseinimehr, and Shahabimajd, 2006).

Antioxidant in food may be defined as any substance which is capable of delaying, retarding or preventing the development of rancidity or other flavor deterioration due to oxidation in two ways. These two ways are by scavenging free radicals (primary antioxidant) or by a mechanism that does not involve direct scavenging of free radicals like binding of metal ions, scavenging oxygen, converting hydroperoxides to non-radical species, absorbing UV radiation or deactivating singlet oxygen (secondary antioxidants) (Gordon, 2001a).

Antioxidants can be found in many kinds of foods. Legumes like peanuts and peas

have dominantly amount of phenolics and phenolic acids. Sterols are antioxidants which can be found in oilseed. In animal product, peptides, amino acids, and carotenoids could potentially serve as natural antioxidants (Hall, 2001). Moreover, antioxidants can also be found in vegetables, fruits, herbs, spices and teas. Especially herbs and spices, are the most important targets in the search for natural antioxidants. Man has used them not only for flavoring foods but also for antiseptic and medical properties since the prehistoric era (Yanishlieva-Maslarova, 2001).

There are many methods to determine the antioxidant activity. Radical-scavenging method is one of the most popular methods because radical scavenging is the main mechanism by which antioxidants act in foods. Antioxidant activities is measured by the scavenging of synthetic radicals like 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzthiazoline-sulphonic acid) (ABTS) in polar organic solvents like methanol. In the DPPH test, the scavenging of DPPH radicals is followed by monitoring the decrease in absorbance at 517 nm (Gordon, 2001b).

Phenolic compounds are the second metabolites that are derivatives of the pentose phosphate, shikimate and phenylpropanoid pathways in plant (Randhir, Lin, and Shetty, 2004). Phenolic compounds exhibit a wide range of physiological properties, such as anti-allergenic, anti-atherogenic, anti-inflammatory, antimicrobial, antioxidant, anti-thrombotic, cardioprotective, and vasodilatory effects (Aberoumand and Deokule, 2008).

Folin-Ciocalteu method is the colorimetric assay of measuring phenolic antioxidants and polyphenol antioxidants. It measures the amount of the substance which inhibits the oxidation of the reagent. This also means that total reducing capacity is measured (Ikawa, 2003).

Thermal processing caused the depletion of antioxidant activity and total phenolic compounds (Roy *et al.*, 2007). According to Roy and coworkers (2007), heating vegetable juices strongly affects their antioxidant and phenolic contents.

Chapter III

Methodology

3.1 Sample Preparation

Four dried herbs, *Imperata cylindrical* (L.) P. Beauv., *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy, *Hedyotis corymbosa* Lamk., and *Orthosiphon aristatus* Miq., were bought from market in China Town, Bangkok, Thailand and kept in glass jar at room temperature.

3.2 Chemical Analysis of Dried Herbs

Each herb was taken to be analyzed for chemical component as followed:

3.2.1 Moisture content (AOAC, 2006) section 30.1.32 (Appendix A.1)

3.2.2 Protein content (AOAC, 2006) section 30.1.33 (Appendix A.2)

3.2.3 Fat content (AOAC, 2006) section 31.4.02 (Appendix A.3)

3.2.4 Crude fiber content (AOAC, 2006) section 30.1.31 (Appendix A.4)

3.2.5 Ash content (AOAC, 2006) section 30.1.25 (Appendix A.5)

3.2.6 Carbohydrate content by the subtraction of each component from 100% (Appendix A.6)

3.2.7 Potassium content (AOAC, 2006) section 50.1.15 (Appendix A.7)

The analyses were performed in three replicates.

3.3 Effect of Infusion Time and Temperature on Herbal Infusion

Each herb infusion was obtained by extracting the herb with water at controlled temperature of 70 °, 80 °, and 90 °C at the ratio of herb:water 1:49 (w/w). The content was covered with aluminum foil and the temperature was controlled in a water bath for 30, 45, and 60 minutes. The infusions then were filtered through a tea filter and the physical and chemical characteristics were measured as followed:

3.3.1 Color (Y, x, y) using colorimeter (Minolta colorimeter CR-300, Japan)

3.3.2 pH using pH meter (Cyberscan pH1000, Singapore)

3.3.3 Total soluble solid using hand refractometer (ATAGO, Japan)

3.3.4 Antioxidant activity as DPPH radical scavenging activity (Sakanaka, Tachibana, and Okada, 2005) (Appendix A.8)

3.3.5 Ascorbic acid content (Pearson, 1976) (Appendix A.9)

3.3.6 Phenolic compounds content (Nawaz *et al.*, 2006) (Appendix A.10)

3.3.7 Potassium content (AOAC, 2006) section 50.1.15 (Appendix A.7)

All analyses were run in triplicates. The experiment was conducted in 3X3 factorial in CRD (completely randomized design). Statistical analysis was carried out by using SPSS (Statistical Package for the Social Science) 15.0 for Windows. The Duncan's new multiple range test was used as the method for multiple comparisons. The optimum condition was determined by response surface method (RSM) of antioxidant activity, ascorbic acid content, total phenolic compounds and potassium content using Design Expert 7.0. The experiments were carried out in 3 replicates.

3.4 Development of Mixed Herbal Drink Formula

3.4.1 Determination of the suitable sugar level in each herbal infusion for sensory evaluation.

Since the herbal infusion was bitter so sugar was added for the sensory evaluation. Each herbal infusion was prepared according to the infusion condition chosen from previous step. Sugar was dissolved in the sample at the level 5, 6, and 7% (w/v). The acceptance of each sweetened herbal infusion at room temperature was evaluated by 30 panelists using 9-point hedonic scale (Appendix B.1). The optimum sugar level for the sample was chosen from the highest liking score.

3.4.2 Evaluation of the bitterness of the herbal infusion.

Each sweetened herbal drink was prepared with optimum sugar level added and evaluated at room temperature for preference by 30 panelists using line scale (Appendix B.2).

3.4.3 Determination of the formula of mixed herbal drink.

The mildest infusion evaluated from previous step was fixed at 10% of mixed infusion. The ratio of the three most bitter infusions was set from linear simplex lattice

design. Sugar was added according to 3.4.1. Each formula was evaluated at room temperature by 30 panelists using 9-point hedonic scale (Appendix B.1). The optimum combination of herbal infusions was determined from the RSM. Next, the appropriate amount of the mildest infusion in the mixed drink was varied at 5, 10 15, and 20%. Sensory evaluation of the samples at room temperature was performed to select the appropriate level of the mildest infusion by 30 panelists.

3.4.4 Determination of the sugar level in mixed herbal drink. Sugar was dissolved in the herbal drink at the level of 5, 6, 7, and 8% (w/v) of finished herbal infusion. The sugar level in the mixed herbal drink giving the highest liking score from 30 panelists using 9-point hedonic scale (Appendix B.1) was chosen.

In sensory evaluation step, all samples were randomly numbered and served to the panelists at one time at room temperature.

3.5 Determination of the Sterilization Conditions

Mixed herbal drink was prepared according to the formula and condition in the previous steps. The drink was sterilized at 130 °, 135 °, and 140 °C to obtain the F_0 of 3, 4, and 5 minutes using a UHT/HTST processing system (Microthermics Bantam-DH, U.S.A.) and filled into 250 milliliters polyethylene terephthalate (PET) bottle in the ultra-clean fill hood (Microthermics Clean Fill Hood, U.S.A.). The samples were then analyzed for

3.5.1 Color (Y, x, y) by using colorimeter (Minolta colorimeter CR-300, Japan)

3.5.2 pH by using pH meter (Cyberscan pH1000, Singapore)

3.5.3 Total soluble solid by using hand refractometer (ATAGO, Japan)

3.5.4 Antioxidant activity as DPPH radical scavenging activity (Sakanaka, Tachibana, and Okada, 2005) (Appendix A.8)

3.5.5 Phenolic compounds content (Nawaz *et al.*, 2006) (Appendix A.10)

3.5.6 Potassium content by (AOAC, 2006) section 50.1.15 (Appendix A.7)

3.5.7 Total plate count by using 3M Petrifilm™

3.5.8 *Clostridium botulinum* (US Food and Drug Administration, 1992) (Appendix A.11)

3.5.9 Sensory quality for acceptability by 30 panelists using 9-point hedonic scale

(Appendix B.1).

All analyses were run in triplicates. The optimum heating condition was determined from the RSM based on the antioxidant activity, total phenolic compounds, potassium content and the amount of *Clostridium botulinum*.

3.6 Changes in Qualities of the Mixed Herbal Drink during Storage

Sterilized mixed herbal drink was prepared according to the formula and condition in the previous steps. It was then filled in PET bottles and kept at room temperature (30 ^{o}C) for four months and at 45 ^{o} and 55 ^{o}C for two months. Samples were taken every week to be analyzed for all properties as in section 3.5 except potassium and *Clostridium botulinum*.

Chapter IV

Results and Discussion

4.1 Chemical Analysis of Dried Herbs

Since, there is no report in chemical constituents of these herbs, so chemical analysis step was conducted. Table 4.1 showed that the *Imperata cylindrical* (L.) P. Beauv. had crude fiber as major component (60.45%). For *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy and *Hedyotis corymbosa* Lamk. contained crude fiber, ash and carbohydrate in the top three amounts at the approximately same level.

Murdannia loriformia (Hassk.) Rolla Rao et Kammathy had the highest amount of potassium, compared to others, followed by *Orthosiphon aristatus* Miq., *Hedyotis corymbosa* Lamk. and *Imperata cylindrical* (L.) P. Beauv., respectively.

Table 4.1 Chemical composition of four dried herbs

Component	Amount (%dry basis)			
	***Imperata cylindrical* (L.) P. Beauv.**	***Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy**	***Hedyotis corymbosa* Lamk.**	***Orthosiphon aristatus* Miq.**
Moisture	17.42 ± 4.25	13.84 ± 2.90	18.92 ± 1.77	14.83 ± 0.73
Protein	1.16 ± 0.02	10.96 ± 0.11	1.84 ± 0.11	8.35 ± 0.05
Fat	0.46 ± 0.39	10.12 ± 0.37	7.68 ± 0.18	5.03 ± 0.34
Crude Fiber	60.45 ± 2.10	24.11 ± 1.46	28.60 ± 2.32	36.11 ± 0.07
Ash	12.13 ± 0.91	28.25 ± 1.38	28.51 ± 1.15	16.52 ± 0.68
Carbohydrate	16.80 ± 3.42	26.56 ± 3.33	33.37 ± 3.76	33.98 ± 1.13
Potassium	0.70 ± 0.02	2.01 ± 0.33	0.93 ± 0.57	1.35 ± 0.35

4.2 Effect of Infusion Time and Temperature on Herbal Infusion

Nine conditions of four herbal infusions were analyzed and shown in Tables 4.2 to 4.5 for physical properties and Tables 4.6 to 4.9 for chemical properties. Tables 4.2 to 4.5 showed that the higher temperature and longer time resulted in darker infusion as shown by Y (brightness), x (red) and y (yellow) values. The result agreed with Kim *et al.* (2007) that increasing extraction time and temperature caused darker tea. Total soluble solid and pH of each herbal drink were slightly different ($p \leq 0.05$).

From Tables 4.6 to 4.9, *Hedyotis corymbosa* Lamk. infusion had the highest antioxidant activity at the mildest condition (70 ^{o}C/30 minutes), followed by *Orthosiphon aristatus* Miq., *Murdannia loriformis* (Hassk.) Rolla Rao et Kammathy and *Imperata cylindrica* (L.) P. Beauv., respectively. However, the antioxidant activity of *Hedyotis corymbosa* Lamk. was dramatically lost when the condition was more severe and thus *Orthosiphon aristatus* Miq. was found to have the highest activity at the same severe condition. In all cases it was found that increasing the infusion time and temperature decrease in antioxidant activity, except *Imperata cylindrica* (L.) P. Beauv. that only infusion time caused the reduction in the antioxidant activity. This may be because most bioactive compound is relatively unstable to heat (Polydera *et al.*, 2004).

The results of the ascorbic acid measurement from Tables 4.6 to 4.9 showed that all herbal infusions contained low level of ascorbic acid. The effects of infusion time and temperature on the ascorbic acid were the same trend as that on the antioxidant activity. A decrease in ascorbic acid during infusion was due to its low thermal resistance (Le?kov? *et al.*, 2006).

From Tables 4.6-4.9, it was found that as the infusion time and temperature increased, the total phenolic compounds increased. *Hedyotis corymbosa* Lamk. had a distinct high amount of phenolic compounds, followed by *Orthosiphon aristatus* Miq., *Murdannia loriformis* (Hassk.) Rolla Rao et Kammathy, and *Imperata cylindrica* (L.) P. Beauv., respectively. When the herbs were soaked in water at various temperatures, the phenolic compounds would leach out from the tissue. The more severe condition gives higher efficiency for phenolic compounds to leach out (Katalinic *et al.*, 2006).

Table 4.2 Physical properties of *Imperata cylindrical* (L.) P. Beauv. infusion

Temperature (°C)	Time (minutes)	Color Value			Total Soluble Solids (°Brix)	pH
		Y	x	y		
70	30	$96.51^{e} \pm 0.19$	$0.3146^{a} \pm 0.0001$	$0.3216^{a} \pm 0.0002$	$0.0^{a} \pm 0.0$	$5.09^{c} \pm 0.04$
	45	$93.93^{bc} \pm 0.39$	$0.3172^{c} \pm 0.0006$	$0.3237^{c} \pm 0.0010$	$0.0^{a} \pm 0.1$	$5.28^{fg} \pm 0.02$
	60	$91.66^{a} \pm 0.83$	$0.3197^{g} \pm 0.0005$	$0.3245^{d} \pm 0.0005$	$0.2^{b} \pm 0.1$	$5.27^{fg} \pm 0.03$
80	30	$95.58^{d} \pm 0.26$	$0.3160^{b} \pm 0.0003$	$0.3230^{b} \pm 0.0003$	$0.0^{a} \pm 0.0$	$5.20^{e} \pm 0.07$
	45	$95.20^{d} \pm 0.44$	$0.3160^{b} \pm 0.0003$	$0.3228^{b} \pm 0.0009$	$0.1^{b} \pm 0.1$	$5.17^{d} \pm 0.08$
	60	$94.47^{c} \pm 0.59$	$0.3159^{b} \pm 0.0007$	$0.3252^{e} \pm 0.0011$	$0.2^{b} \pm 0.1$	$5.29^{g} \pm 0.05$
90	30	$94.03^{bc} \pm 0.28$	$0.3178^{d} \pm 0.0010$	$0.3262^{f} \pm 0.0005$	$0.1^{b} \pm 0.1$	$5.26^{f} \pm 0.04$
	45	$93.39^{b} \pm 0.36$	$0.3186^{e} \pm 0.0005$	$0.3261^{f} \pm 0.0005$	$0.2^{b} \pm 0.0$	$5.00^{a} \pm 0.05$
	60	$92.07^{a} \pm 1.35$	$0.3192^{f} \pm 0.0004$	$0.3286^{g} \pm 0.0012$	$0.2^{b} \pm 0.2$	$5.04^{b} \pm 0.04$

$^{a, b, ..}$ means with different letters within each column are significantly different ($p \leq 0.05$)

Table 4.3 Physical properties of *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy infusion

Temperature (°C)	Time (minutes)	Color Value			Total Soluble Solids (°Brix)	pH
		Y	x	y		
70	30	$75.29^{e} \pm 1.75$	$0.3551^{a} \pm 0.0041$	$0.3635^{a} \pm 0.0038$	$0.2^{b} \pm 0.0$	$5.43^{d} \pm 0.06$
	45	$61.61^{abc} \pm 1.94$	$0.3844^{cd} \pm 0.0074$	$0.3875^{b} \pm 0.0061$	$0.2^{b} \pm 0.1$	$5.47^{d} \pm 0.06$
	60	$55.64^{ab} \pm 15.65$	$0.4010^{d} \pm 0.0391$	$0.3932^{b} \pm 0.0288$	$0.1^{a} \pm 0.1$	$6.03^{e} \pm 0.18$
80	30	$73.89^{de} \pm 3.43$	$0.3571^{ab} \pm 0.0041$	$0.3648^{a} \pm 0.0037$	$0.2^{b} \pm 0.0$	$5.17^{ab} \pm 0.09$
	45	$65.20^{bcd} \pm 11.01$	$0.3773^{bc} \pm 0.0163$	$0.3824^{b} \pm 0.0123$	$0.3^{c} \pm 0.1$	$5.18^{abc} \pm 0.05$
	60	$66.43^{cde} \pm 10.22$	$0.3771^{bc} \pm 0.0151$	$0.3828^{b} \pm 0.0110$	$0.4^{c} \pm 0.0$	$5.14^{a} \pm 0.05$
90	30	$65.09^{bcd} \pm 3.64$	$0.3881^{cd} \pm 0.006$	$0.3924^{b} \pm 0.0047$	$0.2^{b} \pm 0.1$	$5.25^{bc} \pm 0.01$
	45	$53.26^{a} \pm 3.44$	$0.3977^{cd} \pm 0.0076$	$0.3970^{b} \pm 0.0054$	$0.4^{c} \pm 0.1$	$5.21^{abc} \pm 0.05$
	60	$56.43^{ab} \pm 2.21$	$0.3931^{cd} \pm 0.0043$	$0.3937^{b} \pm 0.0030$	$0.5^{d} \pm 0.1$	$5.27^{c} \pm 0.09$

$^{a, b, ..}$ means with different letters within each column are significantly different ($p \leq 0.05$)

Table 4.4 Physical properties of *Hedyotis corymbosa* Lamk. infusion

Temperature (°C)	Time (minutes)	Color Value			Total Soluble Solids (°Brix)	pH
		Y	x	y^{ns}		
70	30	$76.91^{e} \pm 0.66$	$0.3662^{a} \pm 0.0085$	0.3779 ± 0.0094	$0.3^{a} \pm 0.1$	$5.16^{d} \pm 0.10$
	45	$65.26^{d} \pm 4.94$	$0.3696^{a} \pm 0.0106$	0.4224 ± 0.1512	$0.3^{a} \pm 0.1$	$5.16^{d} \pm 0.03$
	60	$65.74^{d} \pm 8.03$	$0.3678^{a} \pm 0.0163$	0.3690 ± 0.0118	$0.3^{a} \pm 0.1$	$5.29^{e} \pm 0.02$
80	30	$62.70^{cd} \pm 4.46$	$0.4124^{d} \pm 0.0141$	0.4149 ± 0.0100	$0.3^{a} \pm 0.1$	$5.27^{e} \pm 0.04$
	45	$60.01^{bc} \pm 6.82$	$0.3735^{a} \pm 0.0123$	0.3783 ± 0.0102	$0.3^{a} \pm 0.1$	$5.57^{f} \pm 0.03$
	60	$56.39^{b} \pm 2.90$	$0.3833^{b} \pm 0.0075$	0.3832 ± 0.0051	$0.3^{a} \pm 0.1$	$4.95^{b} \pm 0.07$
90	30	$49.64^{a} \pm 1.05$	$0.4049^{cd} \pm 0.0016$	0.4000 ± 0.0009	$0.3^{a} \pm 0.1$	$5.18^{d} \pm 0.03$
	45	$50.28^{a} \pm 7.53$	$0.3962^{c} \pm 0.0200$	0.3902 ± 0.0125	$0.4^{b} \pm 0.1$	$4.81^{a} \pm 0.09$
	60	$46.76^{a} \pm 3.28$	$0.4031^{cd} \pm 0.0089$	0.3943 ± 0.0044	$0.6^{c} \pm 0.1$	$5.03^{c} \pm 0.03$

$^{a, b, ..}$ means with different letters within each column are significantly different ($p \leq 0.05$)

Table 4.5 Physical properties of *Orthosiphon aristatus* Miq. infusion

Temperature (°C)	Time (minutes)	Color Value			Total Soluble Solids (°Brix)	pH
		Y^{ns}	x	y		
70	30	72.57 ± 3.93	0.3674^{a} ± 0.0087	0.3770^{ab} ± 0.0092	0.4^{b} ± 0.1	6.09^{e} ± 0.12
	45	66.76 ± 2.08	0.3970^{cd} ± 0.0077	0.4026^{d} ± 0.0057	0.4^{b} ± 0.0	5.58^{c} ± 0.02
	60	64.54 ± 9.63	0.4109^{d} ± 0.0304	0.4099^{d} ± 0.0232	0.4^{b} ± 0.1	5.63^{cd} ± 0.01
80	30	67.02 ± 4.26	0.3690^{a} ± 0.0057	0.3742^{a} ± 0.0051	0.3^{a} ± 0.1	5.37^{a} ± 0.07
	45	72.85 ± 2.13	0.3738^{a} ± 0.0040	0.3858^{bc} ± 0.0040	0.4^{b} ± 0.1	5.57^{c} ± 0.03
	60	72.18 ± 2.36	0.3773^{ab} ± 0.0073	0.3887^{c} ± 0.0067	0.6^{c} ± 0.1	5.60^{cd} ± 0.02
90	30	69.91 ± 2.13	0.3909^{bc} ± 0.0067	0.4005^{d} ± 0.0057	0.3^{a} ± 0.1	5.65^{d} ± 0.03
	45	67.19 ± 1.96	0.3932^{c} ± 0.080	0.3998^{d} ± 0.0064	0.4^{b} ± 0.1	5.42^{ab} ± 0.06
	60	65.96 ± 1.24	0.3960^{c} ± 0.0037	0.4024^{d} ± 0.0031	0.6^{c} ± 0.1	5.46^{b} ± 0.03

$^{a, b, ..}$ means with different letters within each column are significantly different ($p \leq 0.05$)

Table 4.6 Chemical properties of *Imperata cylindrical* (L.) P. Beauv. infusion

Temperature (°C)	Time (minutes)	Antioxidant Activity (mg Trolox eq/ml)	Ascorbic Acid (mg/l)	Total Phenolic compounds (mg gallic acid eq/ml)	Potassium (mg/l)
70	30	$4.55^{e} \pm 1.45$	$2.22^{b} \pm 0.33$	$8.80^{a} \pm 0.47$	$94.02^{a} \pm 12.01$
	45	$2.61^{c} \pm 0.34$	$1.83^{b} \pm 1.10$	$18.53^{b} \pm 0.11$	$109.24^{b} \pm 6.27$
	60	$1.82^{abc} \pm 0.22$	$1.74^{b} \pm 0.58$	$23.21^{d} \pm 0.48$	$120.84^{bc} \pm 11.00$
80	30	$3.97^{de} \pm 0.22$	$0.96^{a} \pm 0.44$	$18.42^{b} \pm 0.01$	$108.04^{b} \pm 11.21$
	45	$2.49^{c} \pm 0.07$	$0.87^{a} \pm 0.50$	$21.33^{c} \pm 0.05$	$121.15^{bc} \pm 9.63$
	60	$1.57^{ab} \pm 0.09$	$0.58^{a} \pm 0.00$	$31.05^{e} \pm 0.01$	$137.48^{d} \pm 10.98$
90	30	$3.57^{d} \pm 0.85$	$0.67^{a} \pm 0.44$	$21.71^{c} \pm 0.02$	$132.62^{cd} \pm 29.13$
	45	$2.18^{bc} \pm 0.20$	$0.58^{a} \pm 0.00$	$23.18^{d} \pm 1.02$	$151.08^{e} \pm 14.85$
	60	$1.27^{a} \pm 0.17$	$0.48^{a} \pm 0.33$	$33.87^{f} \pm 0.01$	$154.56^{e} \pm 2.54$

$^{a, b, ..}$ means with different letters within each column are significantly different ($p \leq 0.05$)

Table 4.7 Chemical properties of *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy infusion

Temperature (°C)	Time (minutes)	Antioxidant Activity (mg Trolox eq/ml)	Ascorbic Acid (mg/l)	Total Phenolic compounds (mg gallic acid eq/ml)	Potassium^{ns} (mg/l)
70	30	$30.43^{e} \pm 3.20$	$2.51^{d} \pm 0.73$	$25.52^{a} \pm 0.01$	507.42 ± 59.24
	45	$7.28^{c} \pm 0.18$	$2.22^{cd} \pm 0.17$	$28.36^{d} \pm 0.10$	562.29 ± 59.64
	60	$4.04^{ab} \pm 0.07$	$1.45^{b} \pm 1.26$	$33.98^{f} \pm 0.01$	591.93 ± 71.32
80	30	$10.16^{d} \pm 4.02$	$1.64^{bc} \pm 0.17$	$26.75^{b} \pm 0.04$	538.78 ± 103.32
	45	$6.64^{bc} \pm 1.55$	$1.45^{b} \pm 0.58$	$30.46^{e} \pm 0.15$	582.05 ± 102.75
	60	$2.91^{a} \pm 0.16$	$1.25^{ab} \pm 0.67$	$40.60^{h} \pm 0.03$	637.73 ± 88.80
90	30	$4.43^{ab} \pm 0.08$	$1.22^{ab} \pm 0.39$	$27.80^{c} \pm 0.01$	601.27 ± 115.78
	45	$3.45^{a} \pm 0.55$	$0.67^{a} \pm 0.17$	$39.63^{g} \pm 0.01$	641.41 ± 179.89
	60	$2.52^{a} \pm 0.15$	$0.58^{a} \pm 0.29$	$43.93^{i} \pm 0.16$	654.31 ± 74.71

$^{a, b, ..}$ means with different letters within each column are significantly different ($p \leq 0.05$)

Table 4.8 Chemical properties of *Hedyotis corymbosa* Lamk. infusion

Temperature (°C)	Time (minutes)	Antioxidant Activity (mg Trolox eq/ml)	Ascorbic Acid^{ns} (mg/l)	Total Phenolic compounds (mg gallic acid eq/ml)	Potassium (mg/l)
70	30	$41.98^{d} \pm 4.26$	1.74 ± 0.29	$58.85^{a} \pm 0.02$	$185.81^{a} \pm 46.73$
	45	$4.73^{b} \pm 1.20$	1.45 ± 1.04	$89.90^{c} \pm 0.01$	$202.33^{a} \pm 125.54$
	60	$2.48^{ab} \pm 0.37$	0.96 ± 0.93	$132.93^{f} \pm 0.02$	$271.39^{b} \pm 34.82$
80	30	$26.53^{c} \pm 3.39$	1.06 ± 0.73	$70.89^{b} \pm 0.02$	$293.13^{bc} \pm 5.94$
	45	$2.98^{ab} \pm 0.15$	0.87 ± 0.29	$116.63^{e} \pm 0.03$	$312.46^{bc} \pm 63.81$
	60	$2.05^{ab} \pm 0.05$	0.67 ± 0.33	$148.91^{g} \pm 0.21$	$360.90^{c} \pm 23.17$
90	30	$4.46^{ab} \pm 0.87$	0.96 ± 0.33	$102.67^{d} \pm 0.05$	$328.93^{bc} \pm 49.30$
	45	$2.22^{ab} \pm 0.15$	0.67 ± 0.17	$150.22^{h} \pm 0.08$	$340.98^{c} \pm 23.93$
	60	$1.47^{a} \pm 0.18$	0.58 ± 0.29	$183.77^{i} \pm 0.07$	$359.03^{c} \pm 16.88$

$^{a, b, ..}$ means with different letters within each column are significantly different ($p \leq 0.05$)

Table 4.9 Chemical properties of *Orthosiphon aristatus* Miq. infusion

Temperature (°C)	Time (minutes)	Antioxidant Activity (mg Trolox eq/ml)	Ascorbic Acid (mg/l)	Total Phenolic compounds (mg gallic acid eq/ml)	Potassium^{ns} (mg/l)
70	30	$38.23^{d} \pm 9.50$	$2.31^{d} \pm 0.58$	$45.81^{a} \pm 0.06$	217.00 ± 69.76
	45	$8.00^{a} \pm 2.21$	$2.22^{d} \pm 0.44$	$50.89^{c} \pm 0.02$	272.19 ± 23.33
	60	$5.54^{a} \pm 1.02$	$2.12^{d} \pm 0.88$	$51.02^{d} \pm 0.01$	293.38 ± 21.72
80	30	$28.08^{c} \pm 4.29$	$1.35^{c} \pm 0.93$	$50.10^{b} \pm 0.17$	265.34 ± 14.37
	45	$5.09^{a} \pm 0.80$	$0.87^{ab} \pm 0.29$	$56.34^{e} \pm 0.01$	$277.04^{b} \pm 7.39$
	60	$4.19^{a} \pm 0.16$	$0.67^{ab} \pm 0.33$	$64.39^{g} \pm 0.02$	303.77 ± 27.37
90	30	$22.65^{b} \pm 0.52$	$1.06^{bc} \pm 0.60$	$51.08^{d} \pm 0.05$	282.37 ± 9.99
	45	$3.32^{a} \pm 0.23$	$0.67^{ab} \pm 0.33$	$58.95^{f} \pm 0.02$	292.64 ± 21.72
	60	$2.86^{a} \pm 0.36$	$0.48^{a} \pm 0.17$	$69.81^{h} \pm 0.01$	331.95 ± 111.72

$^{a, b, ..}$ means with different letters within each column are significantly different ($p \leq 0.05$)

The contrary results of antioxidant activity and total phenolic compounds may be due to method of determination. The DPPH assay determines free antioxidants in the samples and is sensitive to various antioxidants having fast or intermediate kinetic interactions while the Folin-Ciocalteau method determines both free and bound phenolic compounds and is sensitive to wide range of substrates being easily oxidized. Some phenolic compounds such as gallic acid, tannic acid have slow kinetic reactions, so it cannot be detected in this assay. In addition some phenolic antioxidants, such as phenol, coumaric acid, strongly react to Folin-Ciocalteau reagent but do not react to DPPH. Finally, other compounds with absorbance at 517 nm may interfere and lead to underestimate of antioxidant activity (Yang *et al.*, 2007).

Figures 4.1-4.8 illustrate the response surface and overlay plots of these chemical constituents as function of infusion conditions for each herb. The condition for choosing optimum condition was the condition which gave high amount of chemical constituents. Table 4.10 displays the relationships between each chemical constituent as a function of infusion time and temperature of each herb.

From the overlay plots (Figure 4.2, 4.4, 4.6, 4.8) the best condition and the estimated amount of antioxidant activity, ascorbic acid, total phenolic compounds and potassium are tabulated in Table 4.11.

The optimum temperature and time to extract the chemical constituents from the four herbs were 72.1 ^{o}-79.7 ^{o}C and 35.3 - 46.8 minutes. For the industrial scale that uses one big boiler, the average condition has to be found. The average temperature and time of 74.6 ^{o}C and 41 minutes was chosen to prepare the herbal infusion for next step.

4.3 Formulation of Mixed Herbal Drink

Each herbal infusion prepared by extracting the herb with water in ratio 1:49 (w/w) at 74.6 ^{o}C for 41 minutes was determined for suitable sugar level. The bitterness of the herbal infusion was then tasted and the infusions were divided according to their bitterness. Finally, the ratio of herbal drink and sugar were determined.

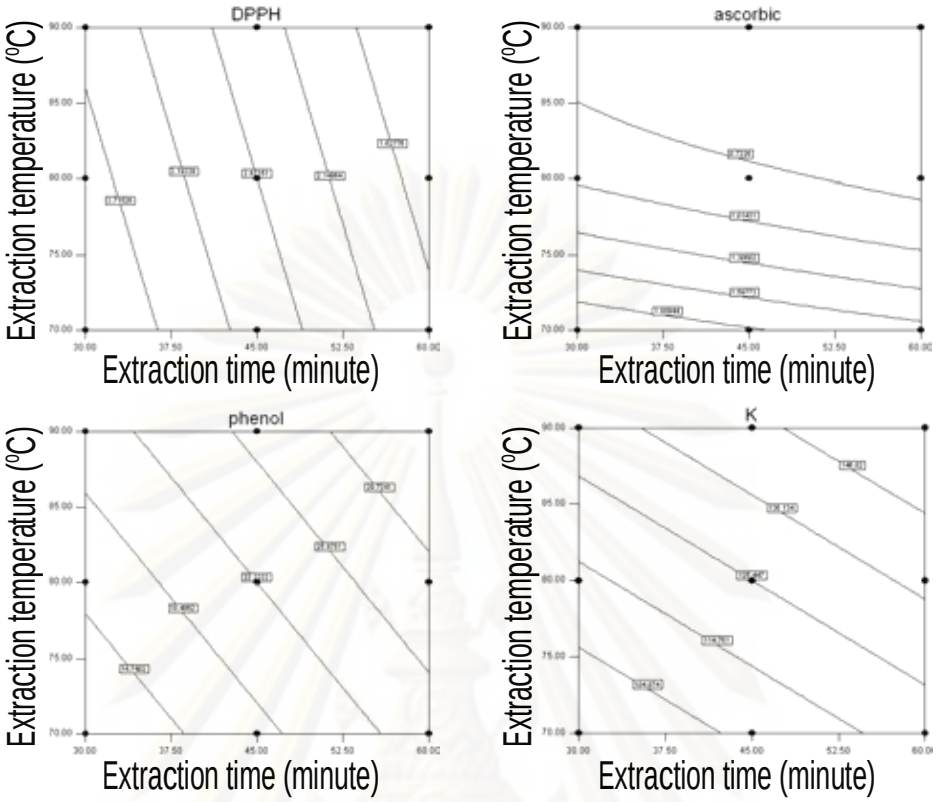

Figure 4.1 Response surfaces of chemical constituents for *Imperata cylindrica* (L.) P. Beauv.

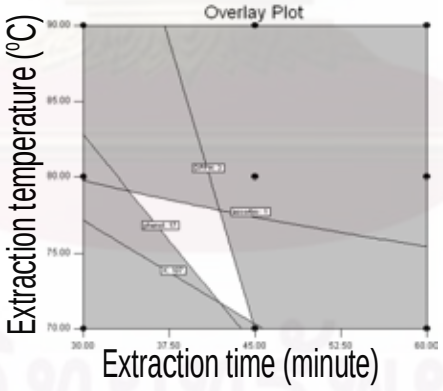

Figure 4.2 Overlay plot of chemical constituents for *Imperata cylindrica* (L.) P. Beauv.

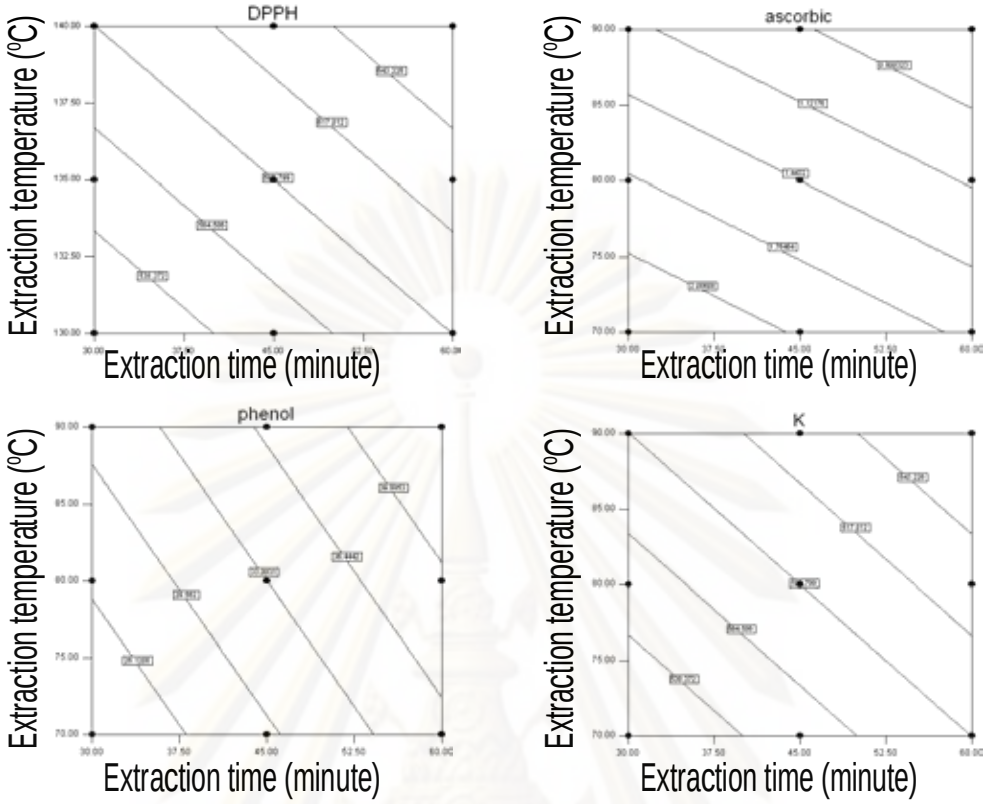

Figure 4.3 Response surfaces of chemical constituents for *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy

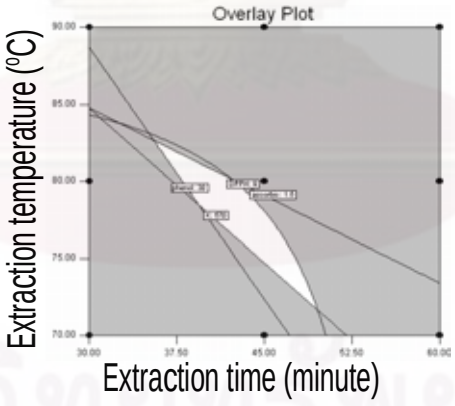

Figure 4.4 Overlay plot of chemical constituents for *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy

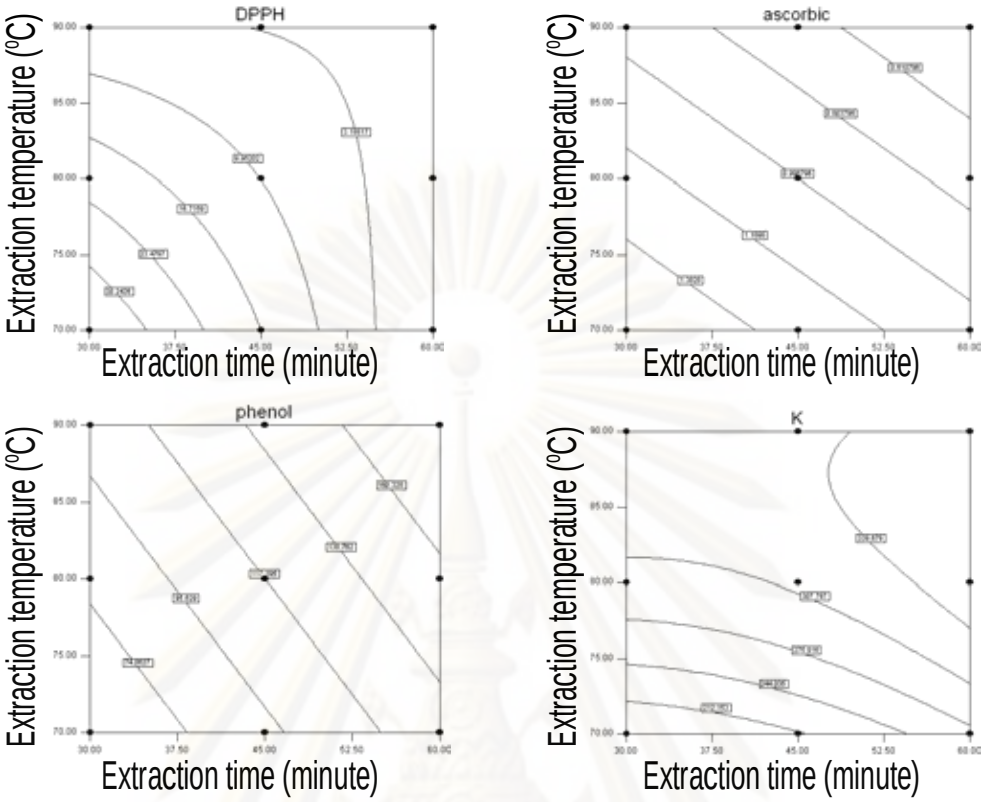

Figure 4.5 Response surfaces of chemical constituents for *Hedyotis corymbosa* Lamk.

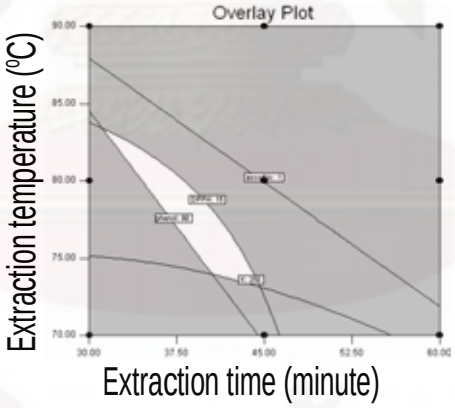

Figure 4.6 Overlay plot of chemical constituents for *Hedyotis corymbosa* Lamk.

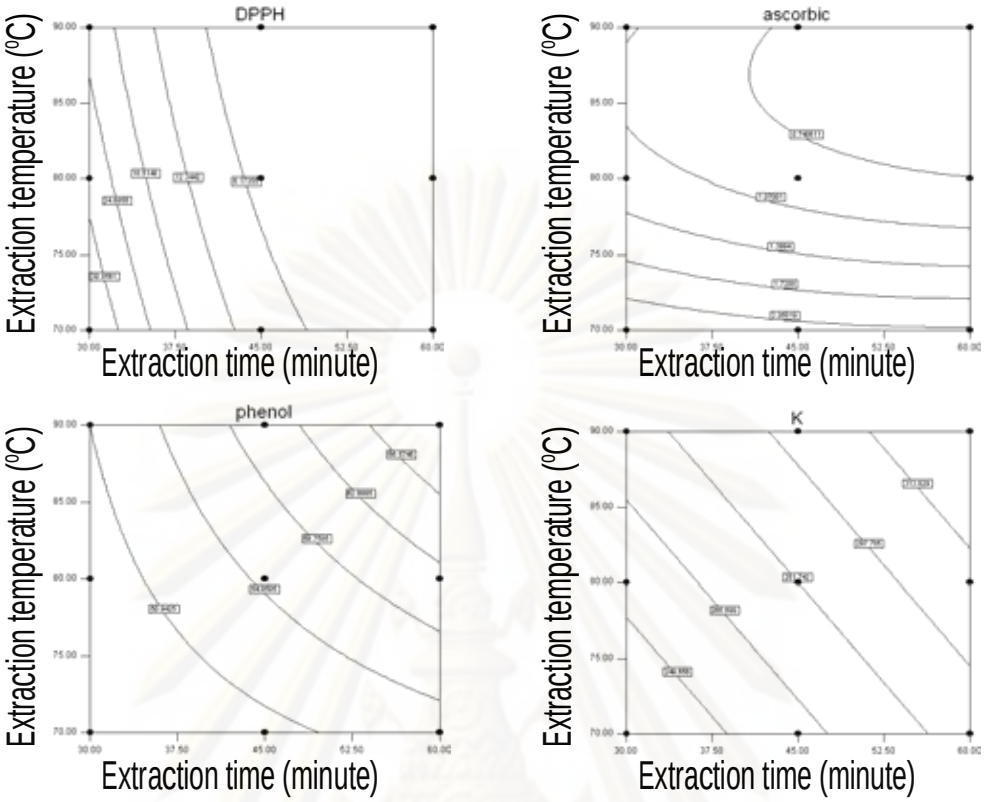

Figure 4.7 Response surfaces of chemical constituents for *Orthosiphon aristatus* Miq.

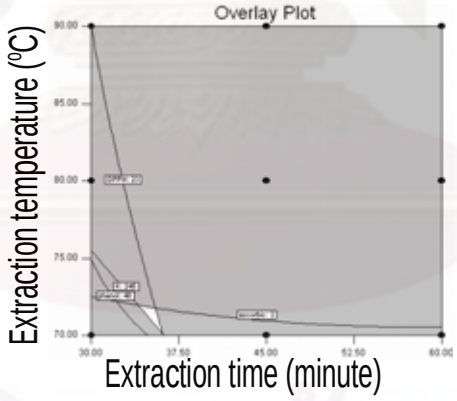

Figure 4.8 Overlay plot of chemical constituents for *Orthosiphon aristatus* Miq.

Table 4.10 Equations express relationship between mixed herbal drink characteristics and time at various storage temperatures

Herbs	Relationship between chemical constituent and infusion time and temperature of each herb	R^2
Imperata cylindrica (L.) P. Beauv.	DPPH=9.0-0.083t-0.033T	0.9648
	ascorbic=37-0.056t-0.81T+4.7x10^{-4}tT+7.2x10^{-4}t^2+4.5x10^{-4}T^2	0.9939
	phenol=-35+0.44t+0.47T	0.9155
	K=-66+0.87t+1.9T	0.9689
Murdannia loriformia (Hassk.) Rolla Rao et Kammathy	DPPH=210-3.7t-2.3T+0.041tT	0.8522
	ascorbic=7.4-0.023t-0.061T	0.9410
	phenol=-17+0.43t+0.39T	0.9090
	K=160+2.6t+3.9T	0.9536
Hedyotis corymbosa Lamk.	DPPH=320-5.6t -3.4T+0.061tT	0.8219
	ascorbic=4.3-0.017t-0.032T	0.8810
	phenol=-210+2.6t+2.6T	0.9839
	K=-3,100+3.6t+76T-0.092tT+0.065t^2-0.41T^2	0.9936
Orthosiphon aristatus Miq.	DPPH=320-7.1t-2.9T+0.022tT+0.051t^2+9.8x10^{-3}T^2	0.9937
	ascorbic=39+3.2x10^{-3}t-0.87T-6.4x10^{-4}tT+3.6x10^{-4}t^2+5.1x10^{-3}T^2	0.9918
	phenol=75-1.4t-0.48T+0.023tT	0.9605
	K=34+1.8t+2.1T	0.9040

where t time (minutes)
T temperature (^{o}C)
DPPH antioxidant activity (mg Trolox eq/ml)
ascorbic ascorbic acid (mg/l)
phenol total phenolic compounds (mg gallic acid eq/ml)
K potassium (mg/l)

Table 4.11 Optimum conditions and predicted antioxidant activity, ascorbic acid, total phenolic compounds for herbal infusion

Herbs	Optimum Temperature (°C)	Optimum Time (minutes)	Antioxidant Activity (mg Trolox eq/ml)	Ascorbic Acid (mg/l)	Total Phenolic compounds (mg gallic acid eq/ml)	Potassium (mg/l)
Imperata cylindrica (L.) P. Beauv.	72.1	42.3	3.16	1.66	17.30	107.94
Murdannia loriformia (Hassk.) Rolla Rao et Kammathy	75.1	46.8	9.50	1.70	31.83	576.18
Hedyotis corymbosa Lamk.	79.7	36.6	16.45	1.15	94.75	297.92
Orthosiphon aristatus Miq.	71.4	35.3	23.79	2.07	48.51	246.00
Average	74.6	41				

4.3.1. Determination of the suitable sugar level in each herbal infusion for sensory evaluation.

Table 4.12 shows the effect of sugar on liking scores of each herbal drink. From the analysis of variance (Table C.37-C.40), it was found that level of sugar did not affect the acceptability of the panelists ($p > 0.05$). For Hedyotis corymbosa Lamk. drink, 5% sugar resulted in lower average score than the others ($p \leq 0.05$) and the drink with 6% sugar had the highest score. The reason that *Hedyotis corymbosa* Lamk. drink required higher sugar level maybe due to its bitter taste. In order to formulate the mixed drink, the bitterness of each herb was tested.

Table 4.12 Average liking score of each herbal drinks with different sugar level

Sugar (%)	***Imperata cylindrica* (L.) P. Beauv. drink^{ns}**	***Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy drink^{ns}**	***Hedyotis corymbosa* Lamk. drink**	***Orthosiphon aristatus* Miq. drink^{ns}**
5	5.7 ± 1.5	4.5 ± 1.6	1.9^{a} ± 1.0	5.1 ± 1.9
6	6.2 ± 1.1	5.2 ± 1.4	4.6^{b} ± 2.0	5.5 ± 1.5
7	6.0 ± 1.7	4.9 ± 1.8	5.0^{b} ± 2.2	4.8 ± 1.7

$^{a, b, ..}$ means with different letters within each column are significantly different ($p \leq 0.05$) when 1 means dislike extremely and 9 means like extremely

4.3.2 Evaluation of the bitterness of the infusions.

Table 4.13 shows that *Imperata cylindrica* (L.) P. Beauv. drink was the mildest while *Hedyotis corymbosa* Lamk. drink was significantly the most bitter ($p \leq 0.05$). *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy drink and *Orthosiphon aristatus* Miq. drink had about the same bitterness. This agreed with the result in Table 4.12 that the panelists liked *Hedyotis corymbosa* Lamk. drink with more sugar added.

Table 4.13 Average score of bitterness for each herbal drinks with 6% sugar added

Herbal Drink	Average Score of Bitterness
Imperata cylindrica (L.) P. Beauv.	$1.1^{a} \pm 1.3$
Murdannia loriformia (Hassk.) Rolla Rao et Kammathy	$3.8^{b} \pm 2.6$
Hedyotis corymbosa Lamk.	$9.1^{c} \pm 1.1$
Orthosiphon aristatus Miq	$4.0^{b} \pm 2.4$

$^{a, b, ..}$ means with different letters are significantly different ($p \leq 0.05$)

0 means no bitter taste and 10 means extremely bitter.

4.3.3 Determination of the formula of the mixed herbal drinks.

Since *Imperata cylindrica* (L.) P. Beauv. drink had the mildest taste, so the amount of this infusion was fixed at 10%. The rest 90% was a mixture of the other three infusions which were varied using a simplex design as shown in Figure 4.9. The liking score of the mixed drink was shown in Table 4.14. From Figure 4.9, the highest liking score was obtained from RSM. The criteria considered are the liking score of higher than 5 and highest amount of *Hedyotis corymbosa* Lamk. due to its high total phenolic compounds contents and low cost. The optimum combination was at the herbal drink ratio of 39.8% *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy, 31.1% *Hedyotis corymbosa* Lamk., and 19.1% *Orthosiphon aristatus* Miq. and the empirical equation relating the average liking score and the level of herbal drink was shown in equation (1).

$$score = 4.8A + 2.3B + 5.6C + 32AB - 3.3AC - 32BC \quad :R^2 = 0.8868 \quad (1)$$

where score liking score

A %*Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy drink

B %*Hedyotis corymbosa* Lamk. drink

C %*Orthosiphon aristatus* Miq. drink

Figure 4.9 Linear simplex lattice designs of three herbal drink mixtures

Table 4.14 Average liking score of the herbal infusions mixture with 6% sugar level

Formula	Infusion Ratio (%)				Liking score
	***Imperata cylindrical* (L.) P. Beauv.**	***Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy**	***Hedyotis corymbosa* Lamk.**	***Orthosiphon aristatus* Miq**	
1	10	90	0	0	5.0 ± 2.0
2	10	0	90	0	2.4 ± 1.3
3	10	0	0	90	5.7 ± 1.6
4	10	60	15	15	6.3 ± 1.2
5	10	15	60	15	2.6 ± 1.4
6	10	15	15	60	1.3 ± 0.5
7	10	30	30	30	5.1 ± 1.8

Then, *Imperata cylindrical* (L.) P. Beauv. was varied and the result (Table 4.15) showed that the mixed herbal drink containing 15% *Imperata cylindrica* (L.) P. Beauv. infusion and 85% three herbal mixture got the highest score. The scores of all samples were 5 - 6.5 which means the panelists like them. However, the panelists stated that the higher concentration of three herbal infusion (formula 1 and 2) gave bitter samples which were not acceptable. The higher level of *Imperata cylindrica* (L.) P. Beauv. infusion gave a very mild taste which was also not acceptable.

Table 4.15 Average liking score of mixed herbal drinks at different ratio

Formula	Infusion Ratio (%)		Liking score
	Imperata cylindrica (L.) P. Beauv. infusion	Three herbal infusion	
1	5	95	$5.3^{a} \pm 1.6$
2	10	90	$5.2^{a} \pm 1.6$
3	15	85	$6.5^{b} \pm 0.9$
4	20	80	$5.0^{a} \pm 1.0$

$^{a, b, ..}$ means with different letters are significantly different ($p \leq 0.05$)

4.3.4 Determination of the sugar level in the mixed herbal drink.

The sweetness of the mixed herbal drink was investigated by varying the amount of sugar in the drinks. The results showed that the samples containing 7% sugar had dominant higher score than the rest (Table 4.16).

Table 4.16 Effect of sugar level on average liking score of mixed herbal drinks

Formula	Herbal Infusion	Sugar	Liking score
1	95	5	$3.9^{a} \pm 1.4$
2	94	6	$5.7^{c} \pm 1.4$
3	93	7	$6.2^{d} \pm 0.6$
4	92	8	$4.9^{b} \pm 1.0$

$^{a, b, ..}$ means with different letters are significantly different ($p \leq 0.05$)

Thus, the mixed herbal drink for the next experimental step consisted of 14% *Imperata cylindrica* (L.) P. Beauv. infusion, 35% *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy infusion, 27% *Hedyotis corymbosa* Lamk. infusion, 17% *Orthosiphon aristatus* Miq. infusion, and 7% sugar.

4.4 Determination of Sterilization Condition

The mixed herbal drink with the 7% sugar was prepared by extracting all dried herbs (0.30% *Imperata cylindrica* (L.) P. Beauv., 0.75% *Murdannia loriformia* (Hassk.)

Rolla Rao et Kammathy, 0.58% *Hedyotis corymbosa* Lamk., 0.37% *Orthosiphon aristatus* Miq.) with water at the ratio 1:49 (w/w) at 74.6 ^{o}C for 41 minutes. The samples were then sterilized at 130 ^{o} - 140 ^{o}C to obtain F_0 at 3 - 5 minutes. The sterilized time is shown in Table 4.17. The physical, chemical and microbiological properties of the heated samples were determined and the results are shown in Tables 4.18 - 4.21.

Table 4.17 Sterilization time on each temperature and F_0

Temperature (^{o}C)	F_0 (minutes)	Sterilization Time (seconds)
130	3	23.2
	4	31
	5	38.7
135	3	7.4
	4	9.8
	5	12.3
140	3	2.4
	4	3.1
	5	3.9

Table 4.18 showed that the higher F_0 and lower sterilization temperature affected color of the sterilized herbal drink as shown by the reduction of x (red), y (yellow) and an increase of Y (brightness) values. This also coincided with the study of Krapfenbauer *et al.* (2006) on apple juice, which might come from browning reaction (Alper, Bahceci, and Acar, 2005).

From Table 4.19, the antioxidant activity and phenolic compounds decreased as the F_0 increased. Some phenolic compounds and bioactive compounds might be destroyed because of the heat treatment (Polydera *et al.*, 2004; Xu *et al.*, 2007). Potassium was not affected by the sterilization because minerals usually had high heat resistance.

The microbiological test (Table 4.20) revealed that all sterilization conditions were safe as no *Clostridium botulinum* and bacteria was detected.

Table 4.18 Physical properties of mixed herbal drink

Temperature (°C)	F_0 (minutes)	Color Value			Total Soluble Solids^{ns} (°Brix)	pH^{ns}
		Y	x	y		
130	3	$67.69^{f} \pm 0.38$	$0.3715^{d} \pm 0.0002$	$0.3727^{b} \pm 0.0001$	7.0 ± 0.1	5.23 ± 0.01
	4	$60.56^{e} \pm 0.25$	$0.3803^{g} \pm 0.0001$	$0.3833^{g} \pm 0.0001$	7.1 ± 0.2	5.25 ± 0.02
	5	$53.47^{b} \pm 0.38$	$0.3930^{j} \pm 0.0000$	$0.3949^{i} \pm 0.0001$	7.0 ± 0.1	5.25 ± 0.01
135	3	$68.02^{f} \pm 0.40$	$0.3658^{b} \pm 0.0006$	$0.3791^{e} \pm 0.0001$	7.0 ± 0.1	5.24 ± 0.01
	4	$60.62^{e} \pm 0.36$	$0.3757^{f} \pm 0.0007$	$0.3718^{a} \pm 0.0001$	7.0 ± 0.1	5.23 ± 0.02
	5	$52.43^{a} \pm 0.81$	$0.3916^{i} \pm 0.0001$	$0.3862^{h} \pm 0.0003$	7.0 ± 0.1	5.24 ± 0.04
140	3	$69.17^{g} \pm 0.47$	$0.3640^{a} \pm 0.0011$	$0.3715^{a} \pm 0.0009$	7.1 ± 0.1	5.23 ± 0.03
	4	$59.29^{d} \pm 1.14$	$0.3736^{e} \pm 0.0001$	$0.3780^{d} \pm 0.0002$	7.1 ± 0.1	5.25 ± 0.01
	5	$54.20^{c} \pm 0.22$	$0.3818^{h} \pm 0.0002$	$0.3814^{f} \pm 0.0005$	7.1 ± 0.1	5.23 ± 0.02
non-sterilized		$70.46^{h} \pm 0.48$	$0.3667^{c} \pm 0.0004$	$0.3743^{c} \pm 0.0001$	7.1 ± 0.1	5.24 ± 0.01

$^{a, b, ..}$ means with different letters within each column are significantly different ($p \leq 0.05$)

Table 4.19 Chemical properties of mixed herbal drink

Temperature (°C)	F_0 (minutes)	Antioxidant Activity (mg Trolox eq/ml)	Total Phenolic compounds (mg gallic acid eq/ml)	Potassium^{ns} (mg/l)
130	3	$8.44^{cde} \pm 1.81$	$42.52^{d} \pm 3.51$	329.848 ± 2.857
	4	$5.12^{b} \pm 2.48$	$31.75^{b} \pm 5.36$	329.329 ± 1.724
	5	$1.67^{a} \pm 0.46$	$20.13^{a} \pm 2.48$	330.439 ± 5.102
135	3	$9.28^{def} \pm 1.54$	$43.33^{d} \pm 1.80$	330.189 ± 1.806
	4	$6.58^{bc} \pm 2.57$	$30.24^{b} \pm 0.38$	327.164 ± 1.279
	5	$1.83^{a} \pm 0.15$	$20.17^{a} \pm 0.29$	330.072 ± 1.491
140	3	$9.64^{ef} \pm 1.09$	$43.00^{d} \pm 1.30$	327.433 ± 2.183
	4	$7.38^{cd} \pm 0.00$	$35.34^{c} \pm 0.56$	327.316 ± 0.739
	5	$2.14^{a} \pm 0.68$	$20.81^{a} \pm 0.71$	330.058 ± 0.288
non-sterilized		$10.87^{f} \pm 3.36$	$45.12^{d} \pm 0.00$	330.579 ± 1.439

$^{a, b, ..}$ means with different letters within each column are significantly different ($p \leq 0.05$)

Table 4.20 Microbiological properties of mixed herbal drink

Temperature (°C)	F_0 (minutes)	Total Plate Count (cfu/ml)	*Clostridium botulinum* (cfu/ml)
130	3	n.d.	n.d.
	4	n.d.	n.d.
	5	n.d.	n.d.
135	3	n.d.	n.d.
	4	n.d.	n.d.
	5	n.d.	n.d.
140	3	n.d.	n.d.
	4	n.d.	n.d.
	5	n.d.	n.d.
non-sterilized		260 ± 30	n.d.

Table 4.21 Average score of mixed herbal drink liking

Temperature (°C)	F_0 (minutes)	Liking Score
130	3	$5.7^{bcd} \pm 1.3$
	4	$5.2^{ab} \pm 1.5$
	5	$7.1^{e} \pm 1.2$
135	3	$6.3^{d} \pm 0.4$
	4	$4.7^{a} \pm 0.4$
	5	$5.5^{bc} \pm 1.1$
140	3	$5.6^{bc} \pm 2.1$
	4	$4.7^{a} \pm 0.8$
	5	$6.1^{cd} \pm 1.0$

$^{a, b}$ means with different letters are significantly different ($p \leq 0.05$)

From liking score (Table 4.21), each condition was not statistically different except the sample heated at 130 ^{o}C and F_0 of 5 minutes which had higher score. Sample heated at 140 ^{o}C and F_0 of 4 minutes had significantly different from the highest score.

Figures 4.10 and 4.11 illustrate the response surface and overlay plots of the antioxidant activity, total phenolic compound and average liking score as function of sterilization conditions for mixed herbal drink. Equations 2-4 expressed the relationship between each quality as a function of sterilization temperature and F_0.

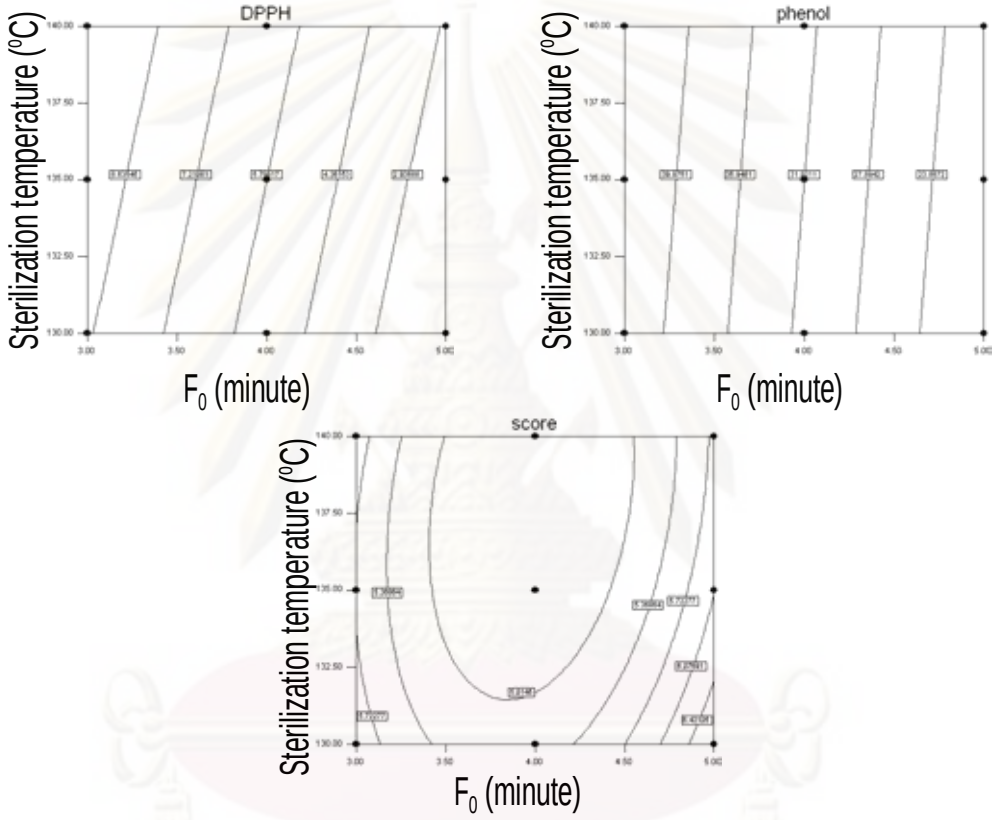

Figure 4.10 Response surfaces of chemical constituents and preference of herbal drink

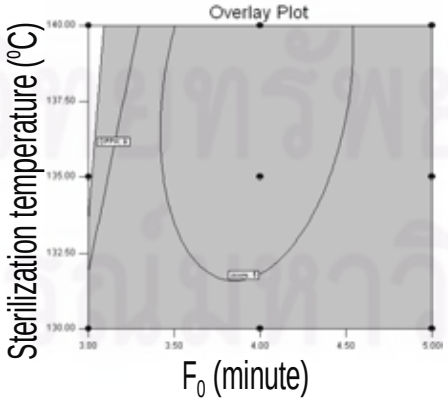

Figure 4.11 Overlay plot of chemical constituents and preference of herbal drink

$DPPH = 2.6 - 3.6F_0 + 0.13T$: $R^2 = 0.9712$ (2)

$phenol = 56 - 11F_0 + 0.16T$: $R^2 = 0.9848$ (3)

$score = 170 - 2.6F_0 - 2.3T - 0.047F_0T + 1.1F_0^2 + 8.9x10^{-3}T^2$: $R^2 = 0.7860$ (4)

where T temperature (^oC)

F_0 lethality value (minute)

DPPH antioxidant activity (mg Trolox eq/ml)

phenol total phenolic compounds (mg gallic acid eq/ml)

score average liking score

From the overlay plot (Figure 4.11) of mixed herbal drink, it was found that the optimum sterilization condition, which be gained from maximizing the chemical constituents and liking score, was heating at 135.4 ^oC with F_0 of 3 minutes. This heating condition gave mixed herbal drink having antioxidant activity of 9.45 mg Trolox eq/ml, total phenolic compounds of 43.25 mg gallic acid eq/ml, and averge liking score of 5.7.

4.5 Changes in qualities of the mixed herbal drink during storage

Changes in qualities of the sterilized product kept at 30 ^oC for four months and at 45 ^{o} and 55 ^oC for two months are shown in Tables 4.22 - 4.28. It was found that total soluble solids and pH of the sterilized mixed herbal drink did not change with storage conditions ($p > 0.05$). While the color of the samples became darker as the storage time increased. This may be due to oxidation of antioxidant compound or precipitation of the pigments (Sandi *et al.*, 2004).

Antioxidant activity and total phenolic compounds also reduced with the storage time. The higher storage temperature caused higher loss on antioxidant activity and total phenolic compounds. Oruma Patthamakanokporn and coworkers (2008) reported that antioxidant activity of homogenized guava and makiang kept at -20 ^oC decreased as the storage time increased. Makiang also lost its antioxidant activity and total phenolic compounds at 5 ^oC. According to Oszmianksi (2008), antioxidant activity and total phenolic compounds of apple pur?e decomposed during storage at 30 ^oC.

Table 4.22 Physical properties of sterilized mixed herbal drink kept at 30 °C

Week	Color Value			Total soluble solids^{ns} (°Brix)	pH^{ns}
	Y	x	y		
0	$60.38^{k} \pm 0.95$	$0.3856^{c} \pm 0.0007$	$0.3871^{a} \pm 0.0008$	7.0 ± 0.1	5.24 ± 0.01
1	$61.06^{l} \pm 0.41$	$0.3837^{a} \pm 0.0002$	$0.3871^{a} \pm 0.0002$	7.0 ± 0.0	5.24 ± 0.01
2	$60.35^{k} \pm 0.03$	$0.3844^{b} \pm 0.0006$	$0.3881^{b} \pm 0.0002$	7.0 ± 0.1	5.24 ± 0.00
3	$60.07^{k} \pm 0.23$	$0.3866^{d} \pm 0.0002$	$0.3886^{c} \pm 0.0002$	6.9 ± 0.1	5.24 ± 0.01
4	$58.45^{j} \pm 0.01$	$0.3872^{e} \pm 0.0001$	$0.3885^{c} \pm 0.0002$	7.0 ± 0.1	5.23 ± 0.01
5	$57.51^{i} \pm 0.08$	$0.3881^{f} \pm 0.0001$	$0.3888^{c} \pm 0.0001$	6.9 ± 0.1	5.23 ± 0.00
6	$57.41^{hi} \pm 0.02$	$0.3910^{h} \pm 0.0001$	$0.3899^{d} \pm 0.0001$	7.0 ± 0.1	5.23 ± 0.01
7	$57.00^{gh} \pm 0.20$	$0.3904^{g} \pm 0.0001$	$0.3910^{e} \pm 0.0000$	$7.0^{c} \pm 0.1$	5.24 ± 0.00
8	$56.87^{g} \pm 0.04$	$0.3912^{h} \pm 0.0003$	$0.3913^{e} \pm 0.0002$	7.0 ± 0.0	5.24 ± 0.01
9	$55.74^{f} \pm 0.22$	$0.3938^{k} \pm 0.0002$	$0.3912^{e} \pm 0.0002$	7.0 ± 0.1	5.23 ± 0.01
10	$54.50^{de} \pm 0.03$	$0.3918^{i} \pm 0.0000$	$0.3919^{f} \pm 0.0001$	6.9 ± 0.0	5.24 ± 0.01
11	$54.88^{e} \pm 0.01$	$0.3924^{j} \pm 0.0001$	$0.3910^{e} \pm 0.0002$	7.0 ± 0.1	5.23 ± 0.01
12	$52.66^{a} \pm 0.01$	$0.3940^{k} \pm 0.0001$	$0.3911^{e} \pm 0.0001$	7.0 ± 0.1	5.24 ± 0.01
13	$52.91^{a} \pm 0.03$	$0.3978^{l} \pm 0.0000$	$0.3912^{e} \pm 0.0000$	7.0 ± 0.1	5.24 ± 0.01
14	$53.82^{bc} \pm 0.16$	$0.3990^{l} \pm 0.0000$	$0.3922^{f} \pm 0.0002$	7.0 ± 0.1	5.23 ± 0.00

Table 4.22 Physical properties of sterilized mixed herbal drink kept at 30 °C (Cont'd)

Week	Color Value			Total Soluble Solids^{ns}	pH^{ns}
	Y	x	y	(°Brix)	
15	52.99^{a} ± 0.27	0.3989^{l} ± 0.0005	0.3921^{f} ± 0.0002	7.0 ± 0.1	5.24 ± 0.01
16	54.12^{cd} ± 0.15	0.3993^{l} ± 0.0001	0.3932^{g} ± 0.0001	7.0 ± 0.0	5.24 ± 0.01
17	53.61^{b} ± 0.02	0.4023^{m} ± 0.0001	0.3943^{h} ± 0.0004	7.0 ± 0.1	5.24 ± 0.01

$^{a, b}$ means with different letters within each column are significantly different ($p \leq 0.05$)

Table 4.23 Physical properties of sterilized mixed herbal drink kept at 45 °C

Week	Color Value			Total Soluble Solids^{ns}	pH^{ns}
	Y	x	y		
0	$60.38^{g} \pm 0.95$	$0.3856^{a} \pm 0.0007$	$0.3871^{a} \pm 0.0008$	7.0 ± 0.1	5.24 ± 0.01
1	$59.05^{f} \pm 0.09$	$0.3873^{c} \pm 0.0001$	$0.3885^{b} \pm 0.0000$	6.9 ± 0.0	5.23 ± 0.00
2	$57.96^{e} \pm 0.04$	$0.3864^{b} \pm 0.0001$	$0.3896^{c} \pm 0.0001$	7.0 ± 0.1	5.23 ± 0.00
3	$57.63^{e} \pm 0.10$	$0.3866^{b} \pm 0.0001$	$0.3896^{c} \pm 0.0001$	7.0 ± 0.1	5.24 ± 0.01
4	$55.55^{d} \pm 0.02$	$0.3893^{d} \pm 00001$	$0.3903^{d} \pm 0.0000$	7.0 ± 0.1	5.24 ± 0.01
5	$54.84^{c} \pm 0.07$	$0.3910^{e} \pm 0.0001$	$0.3904^{d} \pm 0.0001$	7.0 ± 0.2	5.23 ± 0.00
6	$53.78^{a} \pm 0.13$	$0.3912^{e} \pm 0.0001$	$0.3915^{f} \pm 0.0002$	6.9 ± 0.1	5.23 ± 0.00
7	$54.48^{bc} \pm 0.06$	$0.3934^{f} \pm 0.0000$	$0.3918^{f} \pm 0.0001$	7.0 ± 0.1	5.24 ± 0.01
8	$54.06^{ab} \pm 0.13$	$0.3945^{g} \pm 0.0001$	$0.3933^{g} \pm 0.0001$	7.0 ± 0.1	5.24 ± 0.01

$^{a, b}$ means with different letters within each column are significantly different ($p \leq 0.05$)

Table 4.24 Physical properties of sterilized mixed herbal drink kept at 55 °C

Week	Color Value			Total Soluble Solids^{ns}	pH
	Y	x	y		
0	$60.38^{i} \pm 0.95$	$0.3856^{a} \pm 0.0007$	$0.3871^{a} \pm 0.0008$	7.0 ± 0.1	$5.24^{abc} \pm 0.01$
1	$59.53^{h} \pm 0.06$	$0.3890^{b} \pm 0.0003$	$0.3889^{b} \pm 0.0001$	6.9 ± 0.1	$5.23^{a} \pm 0.00$
2	$58.24^{g} \pm 0.10$	$0.3907^{c} \pm 0.0003$	$0.3901^{c} \pm 0.0002$	7.0 ± 0.1	$5.24^{c} \pm 0.01$
3	$56.21^{f} \pm 0.33$	$0.3903^{c} \pm 0.0004$	$0.3903^{c} \pm 0.0003$	7.0 ± 0.1	$5.23^{a} \pm 0.00$
4	$53.72^{e} \pm 0.02$	$0.3940^{d} \pm 0.0001$	$0.3913^{d} \pm 0.0000$	7.0 ± 0.1	$5.24^{bc} \pm 0.00$
5	$51.17^{d} \pm 0.05$	$0.3955^{e} \pm 0.0003$	$0.3923^{e} \pm 0.0003$	7.0 ± 0.1	$5.23^{a} \pm 0.00$
6	$49.26^{c} \pm 0.20$	$0.3957^{e} \pm 0.0002$	$0.3937^{f} \pm 0.0003$	6.9 ± 0.1	$5.24^{bc} \pm 0.00$
7	$46.92^{b} \pm 0.06$	$0.3979^{f} \pm 0.0002$	$0.3953^{g} \pm 0.0001$	6.9 ± 0.0	$5.23^{ab} \pm 0.01$
8	$44.38^{a} \pm 0.95$	$0.3994^{g} \pm 0.0001$	$0.3968^{h} \pm 0.0001$	6.9 ± 0.1	$5.23^{ab} \pm 0.01$

$^{a, b}$ means with different letters within each column are significantly different ($p \leq 0.05$)

Table 4.25 Chemical properties of sterilized mixed herbal drink kept at 30 °C

Week	Antioxidant Activity (mg Trolox eq/ml)	Total Phenolic Compounds (mg gallic acid eq/ml)
0	$10.25^{j} \pm 0.73$	$42.57^{e} \pm 3.77$
1	$10.03^{ij} \pm 0.27$	$43.90^{e} \pm 2.47$
2	$9.54^{hi} \pm 0.95$	$42.64^{e} \pm 5.54$
3	$9.37^{gh} \pm 0.12$	$36.12^{de} \pm 1.09$
4	$8.82^{fg} \pm 0.09$	$31.59^{cd} \pm 1.94$
5	$8.31^{ef} \pm 0.10$	$29.25^{bcd} \pm 2.83$
6	$7.89^{de} \pm 0.02$	$30.02^{bcd} \pm 5.77$
7	$7.87^{de} \pm 0.12$	$29.72^{bcd} \pm 0.88$
8	$7.48^{bcd} \pm 0.13$	$30.04^{bcd} \pm 5.37$
9	$7.69^{de} \pm 0.18$	$24.66^{abc} \pm 0.76$
10	$7.56^{cd} \pm 0.06$	$25.17^{abc} \pm 7.18$
11	$7.29^{bcd} \pm 0.09$	$24.08^{abc} \pm 3.70$
12	$7.35^{bcd} \pm 0.43$	$23.06^{abc} \pm 4.37$
13	$6.90^{ab} \pm 0.48$	$21.30^{ab} \pm 7.67$
14	$6.53^{a} \pm 0.04$	$22.93^{abc} \pm 1.24$
15	$6.91^{abc} \pm 0.23$	$17.69^{a} \pm 9.24$
16	$6.51^{a} \pm 0.05$	$17.35^{a} \pm 6.10$
17	$6.56^{a} \pm 0.09$	$18.81^{a} \pm 5.05$

$^{a, b}$ means with different letters within each column are significantly different ($p \leq 0.05$)

Table 4.26 Chemical properties of sterilized mixed herbal drink kept at 45 °C

Week	Antioxidant Activity (mg Trolox eq/ml)	Total Phenolic Compounds^{ns} (mg gallic acid eq/ml)
0	$10.25^{f} \pm 0.73$	42.57 ± 3.77
1	$8.90^{e} \pm 0.11$	43.57 ± 9.50
2	$8.55^{e} \pm 0.11$	42.31 ± 9.01
3	$7.80^{d} \pm 0.17$	37.11 ± 11.62
4	$7.29^{c} \pm 0.19$	33.01 ± 6.13
5	$6.80^{b} \pm 0.22$	33.90 ± 7.62
6	$6.21^{a} \pm 0.15$	30.79 ± 5.55
7	$6.02^{a} \pm 0.11$	27.87 ± 3.25
8	$6.01^{a} \pm 0.07$	29.70 ± 4.91

$^{a, b}$ means with different letters within each column are significantly different ($p \leq 0.05$)

Table 4.27 Chemical properties of sterilized mixed herbal drink kept at 55 °C

Week	Antioxidant Activity (mg Trolox eq/ml)	Total Phenolic Compounds (mg gallic acid eq/ml)
0	$10.25^{g} \pm 0.73$	$42.57^{e} \pm 3.77$
1	$8.91^{f} \pm 0.45$	$37.18^{cde} \pm 5.68$
2	$8.30^{e} \pm 0.10$	$39.76^{de} \pm 2.61$
3	$7.50^{d} \pm 0.10$	$34.23^{bcd} \pm 10.21$
4	$7.01^{cd} \pm 0.07$	$30.66^{abc} \pm 5.73$
5	$6.53^{bc} \pm 0.04$	$26.97^{ab} \pm 3.51$
6	$6.02^{ab} \pm 0.08$	$27.75^{ab} \pm 5.05$
7	$6.32^{ab} \pm 0.17$	$23.33^{a} \pm 5.12$
8	$5.87^{a} \pm 0.25$	$22.39^{a} \pm 4.03$

$^{a, b}$ means with different letters within each column are significantly different ($p \leq 0.05$)

Table 4.28 Average liking score of sterilized mixed herbal drink while storage

Week	Liking score		
	30 °C	45 °C	55 °C
0	$5.6^{bc} \pm 0.5$	$5.6^{a} \pm 0.5$	$5.6^{b} \pm 0.5$
1	$5.0^{a} \pm 0.9$	$5.8^{ab} \pm 1.0$	$4.8^{a} \pm 1.3$
2	$5.6^{bc} \pm 1.1$	$6.3^{bc} \pm 1.5$	$6.3^{c} \pm 0.8$
3	$5.2^{ab} \pm 0.9$	$6.5^{cd} \pm 1.1$	$5.6^{b} \pm 1.1$
4	$5.7^{cd} \pm 0.8$	$6.3^{bc} \pm 0.4$	$6.0^{bc} \pm 0.7$
5	$6.0^{d} \pm 1.0$	$6.3^{bc} \pm 0.4$	$6.3^{c} \pm 0.8$
6	$6.6^{e} \pm 1.1$	$6.8^{cd} \pm 0.9$	$7.5^{d} \pm 0.5$
7	$7.0^{f} \pm 0.7$	$6.8^{de} \pm 1.5$	$7.5^{d} \pm 0.5$
8	$7.3^{fg} \pm 0.4$	$7.3^{e} \pm 1.1$	$8.0^{e} \pm 0.7$
9	$7.5^{gh} \pm 0.9$		
10	$7.3^{fg} \pm 1.0$		
11	$7.5^{gh} \pm 0.5$		
12	$8.0^{i} \pm 0.7$		
13	$7.8^{hi} \pm 0.4$		
14	$7.5^{gh} \pm 0.5$		
15	$7.8^{hi} \pm 0.9$		
16	$8.5^{j} \pm 0.5$		
17	$7.8^{hi} \pm 0.9$		

$^{a, b}$ means with different letters within each column are significantly different ($p \leq 0.05$)

The average liking score increased with the storage time (Table 4.28). This may due to familiarity effect (Hirokawa and Yamazawa, 2008). From the microbiological test, there was no microorganism detected in the products throughout the study.

From Figures 4.12 - 4.16, changes in color, antioxidant activity and total phenolic compounds increased with and longer storage time and rate of changes increased with higher temperature. Table 4.29 displayed the equations expressed the relationships between each the characteristics of mixed herbal drinks and storage time at each storage temperature.

From Table 4.29, antioxidant activity and total phenolic compounds will be absolutely loss within 40 weeks and 27 weeks, respectively when keeping at room temperature (30 ^{o}C).

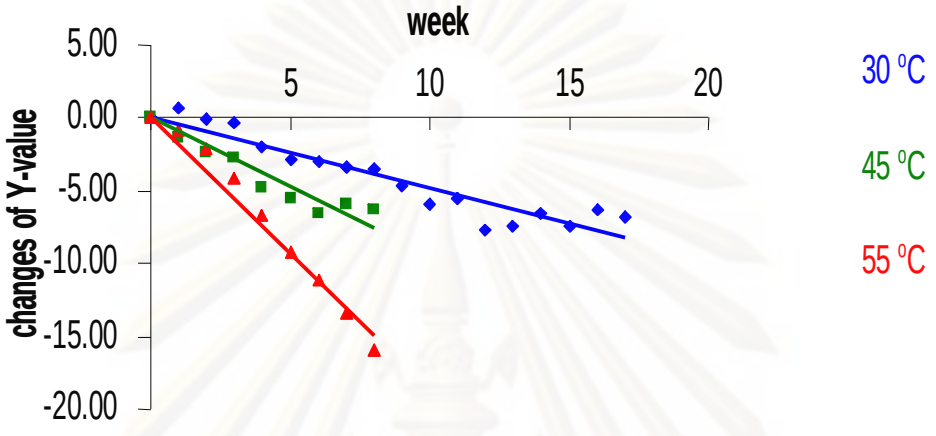

Figure 4.12 Changes of Y-value of sterilized mixed herbal drink during storage

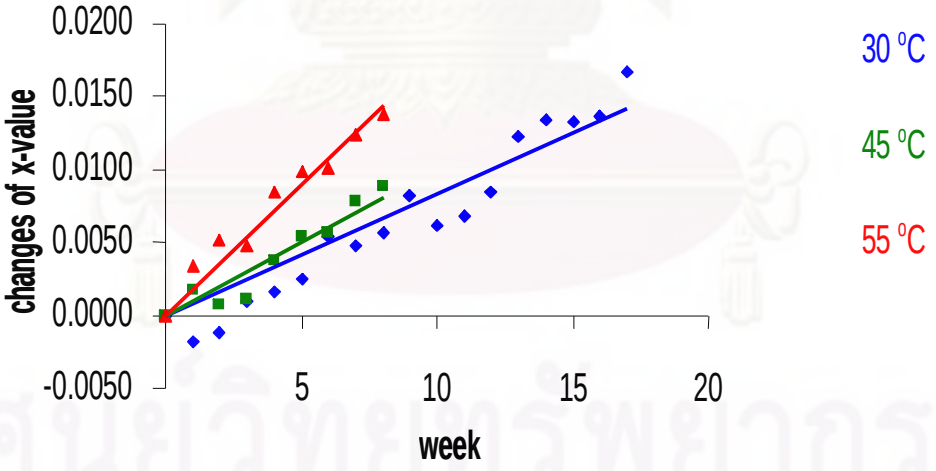

Figure 4.13 Changes of x-value of sterilized mixed herbal drink during storage

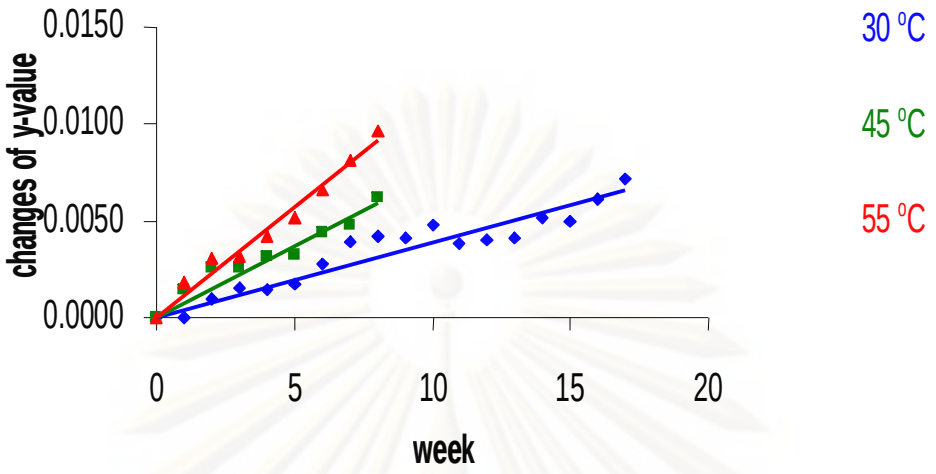

Figure 4.14 Changes of y-value of sterilized mixed herbal drink during storage

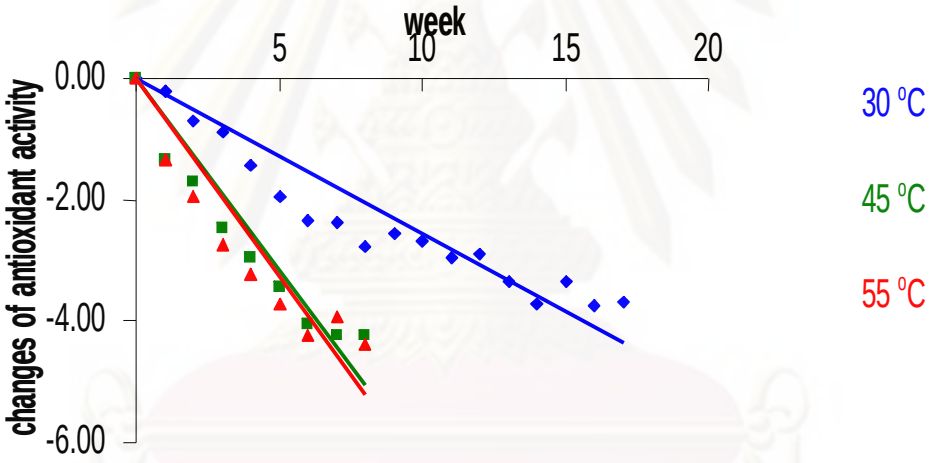

Figure 4.15 Changes of antioxidant activity of sterilized mixed herbal drink during storage

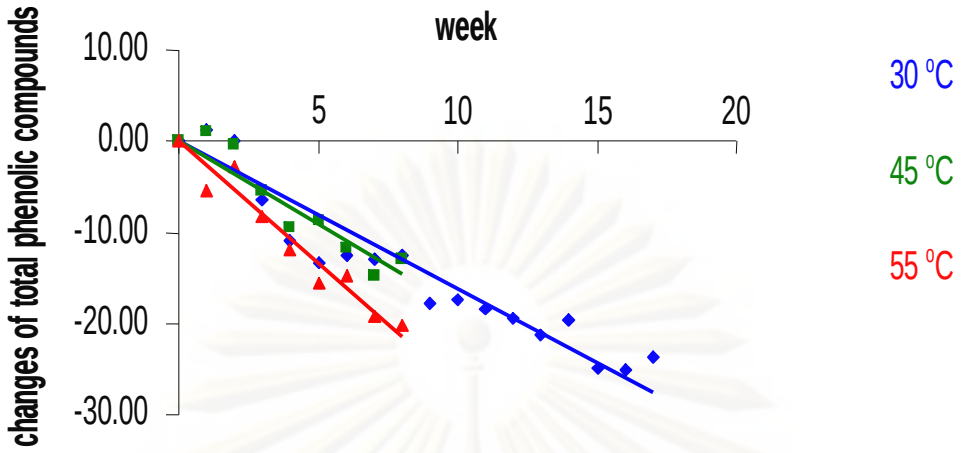

Figure 4.16 Changes of total phenolic compounds of sterilized mixed herbal drink during storage

Table 4.29 Equations express relationship between mixed herbal drink characteristics and time at various storage temperatures

Characteristics (y)	A coeficient in equation* at temperature		
	30 °C	45 °C	55 °C
Y-value	-0.4821t (0.8945)	-0.9459t (0.8890)	-1.8592t (0.9712)
x-value	0.0008t (0.9049)	0.0010t (0.9087)	0.0018t (0.9471)
y-value	0.0004t (0.9043)	0.0007t (0.9199)	0.0011t (0.8109)
DPPH	-0.2567t (0.8709)	-0.6325t (0.8816)	-0.6522t (0.8109)
phenol	-1.6240t (0.9022)	-1.8336t (0.8878)	-2.6796t (0.9431)

*equation: $y = At$

number in parentheses means R^2 value

where t storage time (week)

Y-value changes in Y-value (lightness)

x-value changes in x-value (red)

y-value changes in y-value (yellow)

DPPH changes in antioxidant activity (mg Trolox eq/ml)

phenol changes in total phenolic compounds (mg gallic acid eq/ml)

Chapter V

Conclusion

5.1 Conclusion

From proximate analysis, all four herbs, *Imperata cylindrical* (L.) P. Beauv., *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy, *Hedyotis corymbosa* Lamk., and *Orthosiphon aristatus* Miq., had fiber as a major component and protein and fat as minor components. *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy and *Orthosiphon aristatus* Miq. had high amount of potassium.

In the extraction process, the more severe conditions (high temperature and long time) result in a darker herbal infusion and lower antioxidant activity and ascorbic acid. On the other hand, the amount of total phenolic compounds and potassium increased as the extraction temperature and time increased. The most appropriate extraction condition was at 74.6 ^{o}C for 41 minutes.

The most acceptable formula for mixed herbal infusion composed of 14% *Imperata cylindrical* (L.) P. Beauv. infusion, 35% *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy infusion, 27% *Hedyotis corymbosa* Lamk. infusion, 17% *Orthosiphon aristatus* Miq. infusion, and 7% sugar.

From the sterilization process, heating caused darker products and reduced the antioxidant activity and total phenolic compounds. Total soluble solids, pH and amount of potassium were not affected by the sterilization. There was no microorganism detected in any sterilized mixed herbal drink. The most appropriate sterilization process was 135.4 ^{o}C at F_0 = 3 minutes.

The antioxidant activity and total phenolic compounds of the sterilized mixed herbal drink decreased as the storage time and temperature increased. Antioxidant activity and total phenolic compounds will absolutely loss within 40 weeks and 27 weeks, respectively.

5.2 Suggesion

The familiarity effect during the storage test needs more investigation. The market test and economic analysis of the product are suggested to make sure that this product can be launched and succeed in the market.

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Appendices

Appendix A

Physical, Chemical and Microbiological Analyses

A.1 Moisture analysis by AOAC (2006) section 30.1.32

Instruments

1. Hot air oven (Memmert W350, Germany)
2. Aluminium cup
3. Four-digit digital balance (Mettler Toledo AB204, Switzerland)
4. Desiccator

Methods

1. Weight about 2 g of sample, accurately in a tare previously heated and cooled aluminium cup
2. Place weighted sample plus cup in oven and dry (100 $\pm$ 2 ^{o}C) for about 1.5-2 h
3. Remove and cool in desiccator and weight
4. Calculate by using equation

$$\%\text{moisture} = 100 \times \frac{\text{wt loss on drying, g}}{\text{wt test portion, g}}$$

A.2 Protein analysis by AOAC (2006) section 30.1.33

Instruments

1. Kjeldahl instrument (BUCHI, Switzerland)
2. Four-digit digital balance (Mettler Toledo AB204, Switzerland)

Reagents

1. Concentrated sulfuric acid (A.R. grade)
2. 4% boric acid (A.R. grade)
3. 50% sodium hydroxide (A.R. grade)
4. 0.1 N hydrochloric acid (A.R. grade)
5. Indicator composed of 2 parts of 0.2% alcoholic methyl red solution and 1 part of 0.2% alcoholic methylene blue solution

Methods

1. Transfer approximately 0.8-1.2 g of dried samples, accurately weighed to a digestion tube
2. Add 20 ml concentrated H_2SO_4
3. Place the tube in the preheated digester for about 30 minutes until a clear solution is obtained
4. Remove the tubes from the digester, cool and dilute with 30 ml water
5. Place a conical flask containing 25 ml of 4% boric acid and 2 drops of indicator under the condenser outlet
6. Set up the distillation unit, and program by setting 70 ml of 50% NaOH, 50 ml water, 20 ml of 4% H_3BO_3 for 6 minutes
7. Remove receiving flask and rinse condenser tip into receiving flask using distilled water
8. Titrate contents of receiving flask with 0.1 N HCl until the solution change from green to purplish red
9. Repeat 1-8, but do not put the sample for blank
10. Calculate the amount of protein

$$\%\text{protein} = \frac{0.14?\text{volume of 0.1 M HCl}}{\text{weight of food, g}} \times 6.25$$

A.3 Fat analysis by AOAC (2006) section 31.4.02

Instruments

1. Soxhlet apparatus (Soxtherm Gerhardt S-226, Germany)
2. Hot air oven (Memmert W350, Germany)
3. Four-digit digital balance (Mettler Toledo AB204, Switzerland)
4. Desiccator

Reagents

1. Petroleum ether

Methods

1. Accurately weigh 2 g of dried sample and wrap with Whatman No.1 filter paper before transferring to extraction thimble

2. Plug the end of the thimble with fat-free cotton wool, then place the thimble and content in the central syphon portion of the Soxhlet apparatus
3. Weigh the dried 250 ml flask
4. Pour in 80 ml petroleum ether into the flask and connect it to the Soxhlet syphon and condenser
5. Reflux at a rate of 5 or 6 drops per second condensation for about 4 hours
6. Remove the ether by cautious evaporation of the content of the Soxhlet flask on hot plate
7. Dry in air oven at 100 ^{o}C for 30 minutes, cool.
8. Weigh the flask
9. Calculate the amount of fat from weight increase

A.4 Crude fiber analysis by AOAC (2006) section 30.1.31

Instruments

1. Crucible
2. Hot air oven (Memmert W350, Germany)
3. Muffle furnace (Carbolite CWF 1200, England)
4. Four-digit digital balance (Mettler Toledo AB204, Switzerland)
5. Desiccator
6. Hot Plate

Reagents

1. 1.25% (v/v) sulfuric acid (A.R. grade)
2. 1.25% (w/v) sodium hydroxide (A.R. grade)
3. 95% ethyl alcohol

Methods

1. Transfer defatted sample from fat analysis to 600 ml beaker
2. Add 200 ml of 1.25% H_2SO_4 and heat rapidly and gently for 30 minutes and keep the volume constant by adding hot water time to time
3. Filter the solution through linen on a buchner and wash beaker, linen and residue several times with large amounts of hot water until the filtrate is free from acid
4. Wash the residue from the linen back into beaker with hot water
5. Add 200 ml of 1.25% NaOH and boil for 30 minutes and maintain the volume

6. Filter through the same linen and transfer the residue to the filter by means of a jet of hot water several times until the filtrate is free from base
7. Wash the residue twice with 25 ml of 95% ethyl alcohol
8. Put the residue into the oven at 100 ^{o}C until the weigh is constant
9. Cool down the residue in desiccator and weigh
10. Transfer the residue to crucible, then heat on the hot plate until the smoke is not expelled
11. Place crucible in the furnace at 550 ^{o}C until white ash is obtained
12. Cool down in desiccator and weigh
13. Calculate the amount of crude fiber

$$\%\text{crude fiber} = \left(\frac{\text{sample before ignition, g - ignited sample, g}}{\text{defatted sample, g}} \times 100 \right) \left(1 - \frac{\%\text{lipid}}{100} \right)$$

A.5 Ash analysis by AOAC (2006) section 30.1.25

Instruments

1. Crucible
2. Muffle furnace (Carbolite CWF 1200, England)
3. Four-digit digital balance (Mettler Toledo AB204, Switzerland)
4. Desiccator
5. Hot plate

Methods

1. Transfer the dried sample approximately 3-5 g, weigh accurately, to crucible, then heat on the hot plate until the smoke is not expelled
2. Place crucible in the furnace at 550 ^{o}C until white ash is obtained
3. Cool down in desiccator and weigh
4. Calculate the amount of ash

$$\%\text{ash} = \frac{\text{sample weight after ignition, g}}{\text{dried sample, g}} \times 100$$

A.6 Carbohydrate calculation

$$\%\text{carbohydrate} = 100 - \%(\text{protein} + \text{fat} + \text{crude fiber} + \text{ash})$$

A.7 Potassium analysis by AOAC (2006) section 50.1.15

Instruments

1. Inductively coupled plasma emission spectrometer (Model 975 Plasma Atom Comp, USA)
2. Hot plate

Reagents

1. Concentrated nitric acid (A.R. grade)
2. Concentrated hydrochloric acid (A.R. grade)
3. Potassium standard prepared by dissolving 1.9067 g KCl in water and dilute to 1 litre with deionized water

Methods

1. Pipette 50 ml sample in 100 ml beaker
2. add 5 ml concentrated HNO_3 and 1 ml concentrated HCl
3. Heat on the hot plate without boiling until the volume is about 15-20 ml
4. Cool down and filter through Whatman No. 42 filter paper to 50 ml volumetric flask
5. Rinse the beaker with deionized water three times and pour in the same volumetric flask
6. Make the volume to 50 ml with deionized water
7. Calibrate the ICP using potassium standard, analyze the digested sample with ICP at 766.5 nm

A.8 Antioxidant activity determination by DPPH radical scavenging activity (Sakanaka, Tachibana, and Okada, 2005)

Instruments

1. Spectrophotometer (Spectronic Genesys 20, US)

Reagents

1. 2,2-diphenyl-1-picrylhydrazy
2. methanol (A.R. grade)
3. 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

Methods

1. Add 2.9 ml of 0.1 mM DPPH in methanol and 0.1 ml of sample, keep in dark for 30 minutes
2. Read absorbance at 517 nm
3. Do the same of 1-2 but use methanol instead of sample as control
4. Calculate %inhibition

$$\%\text{inhibition} = \frac{\text{absorbance of control - absorbance of sample}}{\text{absorbance of control}}$$

5. Plot calibration curve by using range of Trolox from 1 to 100 mg/ml instead of sample and do the step 1-4, then plot between log of Trolox concentration and %inhibition

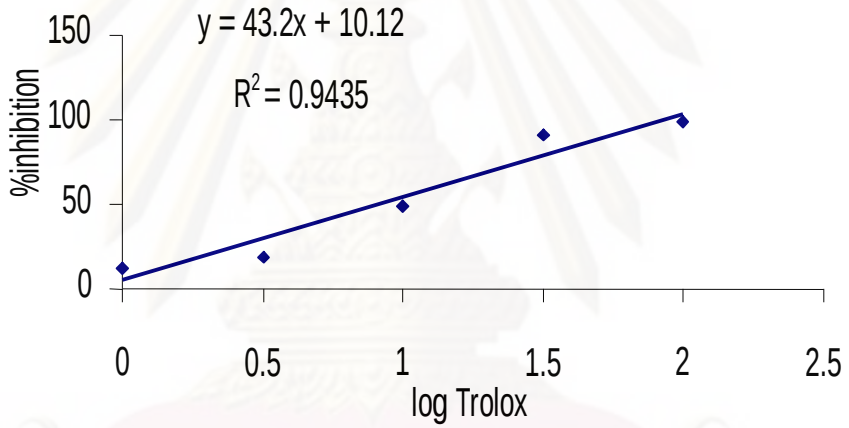


Figure A.1 Trolox standard curve

6. Use the curve to determine antioxidant activity of sample

A.9 Ascorbic acid analysis by photometric method (Pearson, 1976)

Instruments

1. Spectrophotometer (Spectronic Genesys 20, US)

Reagents

1. 0.4% (w/v) oxalic acid (A.R grade)
2. 0.0012% (w/v) 2,6-dichlorophenolindophenol
3. 0.1% (w/v) ascorbic acid in 0.4% oxalic acid

Methods

1. Add 1 ml sample and 9 ml water, set zero absorbance at 520 nm
2. Add 1 ml sample and 9 ml 0.0012% 2,6-dichlorophenolindophenol, read absorbance at 520 nm (L_x)
3. Read absorbance at 517 nm
4. Do the same of 1-2 but use 0.4% oxalic acid instead of sample as control (L_1)
5. Plot calibration curve by using range of ascorbic acid from 0.01 to 0.04 mg/ml instead of sample and do the step 1-4, then plot between L_1 - L_x and ascorbic acid concentration

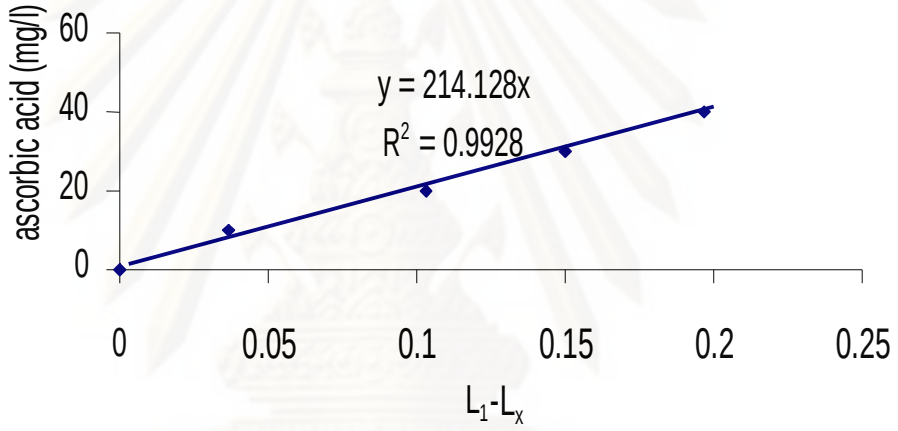


Figure A.2 Ascorbic acid standard curve

6. Use the curve to find ascorbic acid content

A.10 Total phenolic compounds analysis by Folin-Ciocalteau colorimetric method (Nawaz *et al.*, 2006)

Instruments

1. Spectrophotometer (Spectronic Genesys 20, US)

Reagents

1. Gallic acid (A.R grade)
2. Folin-Ciocalteau reagent
3. 7.5% sodium carbonate (A.R. grade)

Methods

1. Add 0.1 ml sample, 0.1 ml F&C reagent and 2 ml water, wait for 5 minutes
2. Add 1.6 ml of 7.5% Na_2CO_3, wait for 2 hours
3. Read absorbance at 765 nm
4. Plot calibration curve by using range of gallic acid from 0.0005 to 0.05 g/ml instead of sample and do the step 1-3, then plot between concentration and absorbance

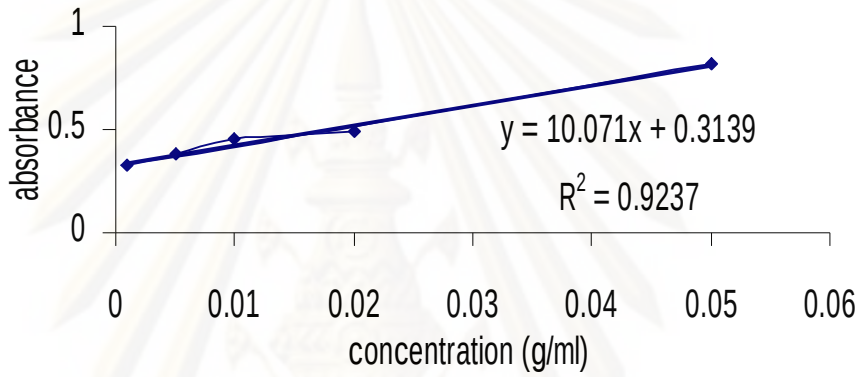


Figure A.3 Gallic acid standard curve

5. Use the curve to find total phenolic content as gallic acid equivalent. If the absorbance does not be covered by graph, dilute the solution and recalculate.

A.11 *Clostridium botulinum* analysis by BAM (1992) method (U.S. Food and Drug Administration, 1992)

Media

1. Cooked meat medium
2. Tryptone peptone glucose yeast extract broth

Methods

1. Remove dissolved oxygen from media by steaming 10-15 minutes and cooling quickly without agitation before inoculation
2. Inoculate 2 tubes of cooked meat medium with 1 ml sample per 15 ml enrichment broth
3. Inoculate 2 tubes of TPGY broth as step 2

4. Incubate at 26 ^{o}C and examine culture by smear stained by Grams reagent, observe *Clostridium botulinum* cell

5. Incubate 10 more days if there is no growth

Appendix B

Sensory Questionnaire

B.1 Preference questionnaire

Name_____Age_____

Gender_____Sample Code_____Date_____

Guidelines

1. Rinse your mouth after tasting each sample
2. Answer all questions

1. Preference of sample by ticking X in the box that most appropriate

Level of Preference	Sample Code			
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				

2. Acceptance of sample by ticking X in the box that most appropriate

   Sample code____ ☐ Accept ☐ Unaccept Reasons_____

   Sample code____ ☐ Accept ☐ Unaccept Reasons_____

   Sample code____ ☐ Accept ☐ Unaccept Reasons_____

   Sample code____ ☐ Accept ☐ Unaccept Reasons_____

3. Comments

_____

B.2 Bitterness questionnaire

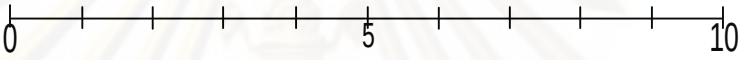
Name_____Age_____

Gender_____Sample Code_____Date_____

Guidelines

1. Rinse your mouth after tasting each sample
2. Answer all questions

1. Bitterness of sample by draw the line that you think most appropriate



Not bitter Very bitter

2. Comments

_____

_____

Appendix C

Statistical Analysis

Table C.1 Analysis of variance of Y in extraction step of *Imperata cylindrical* (L.) P. Beauv. infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	16.635	2	8.318	22.541	.000
Time	31.448	2	15.724	42.613	.000
Temp * Time	11.822	4	2.955	8.010	.001
Rep	.960	2	.480	1.300	.300
Error	5.904	16	.369		
Total	66.769	26			

Table C.2 Analysis of variance of x in extraction step of *Imperata cylindrical* (L.) P. Beauv. infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	5.09E-005	2	2.55E-005	46.650	.000
Time	5.06E-005	2	2.53E-005	46.378	.000
Temp * Time	9.11E-006	4	2.28E-006	4.173	.017
Rep	1.61E-006	2	8.03E-007	1.472	.259
Error	8.73E-006	16	5.46E-007		
Total	.000	26			

Table C.3 Analysis of variance of y in extraction step of *Imperata cylindrical* (L.) P. Beauv. infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	7.39E-005	2	3.69E-005	45.681	.000
Time	3.01E-005	2	1.50E-005	18.599	.000
Temp * Time	5.42E-006	4	1.35E-006	1.676	.205
Rep	1.30E-006	2	6.51E-007	.806	.464
Error	1.29E-005	16	8.09E-007		
Total	.000	26			

Table C.4 Analysis of variance of total soluble solid in extraction step of *Imperata cylindrical* (L.) P. Beauv. infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	.067	2	.034	19.158	.000
Time	.059	2	.029	16.632	.000
Temp * Time	.041	4	.010	5.895	.004
Rep	.005	2	.003	1.474	.259
Error	.028	16	.002		
Total	.201	26			

Table C.5 Analysis of variance of pH in extraction step of *Imperata cylindrical* (L.) P. Beauv. infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	.084	2	.042	13.001	.000
Time	.013	2	.006	1.957	.174
Temp * Time	.200	4	.050	15.452	.000
Rep	.001	2	.000	.134	.876
Error	.052	16	.003		
Total	.350	26			

Table C.6 Analysis of variance of antioxidant activity in extraction step of *Imperata cylindrical* (L.) P. Beauv. infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	1.928	2	.964	2.700	.098
Time	28.378	2	14.189	39.749	.000
Temp * Time	.290	4	.073	.203	.933
Rep	.496	2	.248	.695	.514
Error	5.711	16	.357		
Total	36.803	26			

Table C.7 Analysis of variance of ascorbic acid in extraction step of *Imperata cylindrical* (L.) P. Beauv. infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	9.364	2	4.682	16.440	.000
Time	.560	2	.280	.983	.396
Temp * Time	.122	4	.031	.107	.978
Rep	.229	2	.114	.401	.676
Error	4.557	16	.285		
Total	14.831	26			

Table C.8 Analysis of variance of total phenolic compounds in extraction step of *Imperata cylindrical* (L.) P. Beauv. infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	423.560	2	211.780	1125.511	.000
Time	788.438	2	394.219	2095.088	.000
Temp * Time	62.531	4	15.633	83.080	.000
Rep	.003	2	.001	.007	.993
Error	3.011	16	.188		
Total	1277.542	26			

Table C.9 Analysis of variance of potassium in extraction step of *Imperata cylindrical* (L.) P. Beauv. infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	6656.200	2	3328.100	16.528	.000
Time	3097.010	2	1548.505	7.690	.005
Temp * Time	127.869	4	31.967	.159	.956
Rep	216.270	2	108.135	.537	.595
Error	3221.770	16	201.361		
Total	13319.120	26			

Table C.10 Analysis of variance of Y in extraction step of *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	476.595	2	238.297	3.088	.073
Time	817.272	2	408.636	5.295	.017
Temp * Time	149.479	4	37.370	.484	.747
Rep	129.150	2	64.575	.837	.451
Error	1234.724	16	77.170		
Total	2807.220	26			

Table C.11 Analysis of variance of x in extraction step of *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	.002	2	.001	3.572	.052
Time	.003	2	.001	4.527	.028
Temp * Time	.001	4	.000	1.020	.427
Rep	.001	2	.000	1.350	.287
Error	.005	16	.000		
Total	.012	26			

Table C.12 Analysis of variance of y in extraction step of *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	.002	2	.001	4.293	.032
Time	.002	2	.001	4.316	.032
Temp * Time	.001	4	.000	.915	.479
Rep	.000	2	.000	1.316	.296
Error	.003	16	.000		
Total	.007	26			

Table C.13 Analysis of variance of total soluble solid in extraction step of *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	.165	2	.083	28.774	.000
Time	.081	2	.040	14.065	.000
Temp * Time	.113	4	.028	9.806	.000
Rep	.014	2	.007	2.452	.118
Error	.046	16	.003		
Total	.419	26			

Table C.14 Analysis of variance of pH in extraction step of *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	1.185	2	.592	55.557	.000
Time	.233	2	.117	10.948	.001
Temp * Time	.463	4	.116	10.852	.000
Rep	.000	2	.000	.023	.977
Error	.171	16	.011		
Total	2.052	26			

Table C.15 Analysis of variance of antioxidant activity in extraction step of *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	518.392	2	259.196	71.352	.000
Time	697.236	2	348.618	95.969	.000
Temp * Time	630.177	4	157.544	43.369	.000
Rep	.233	2	.117	.032	.968
Error	58.122	16	3.633		
Total	1904.161	26			

Table C.16 Analysis of variance of ascorbic acid in extraction step of *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	6.784	2	3.392	10.925	.001
Time	2.198	2	1.099	3.540	.053
Temp * Time	.558	4	.139	.449	.772
Rep	1.440	2	.720	2.319	.131
Error	4.967	16	.310		
Total	15.946	26			

Table C.17 Analysis of variance of total phenolic compounds in extraction step of *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	278.317	2	139.159	18218.225	.000
Time	739.280	2	369.640	48392.154	.000
Temp * Time	99.041	4	24.760	3241.547	.000
Rep	.000	2	.000	.025	.975
Error	.122	16	.008		
Total	1116.761	26			

Table C.18 Analysis of variance of potassium in extraction step of *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	27978.853	2	13989.427	1.842	.191
Time	28234.514	2	14117.257	1.858	.188
Temp * Time	2151.746	4	537.936	.071	.990
Rep	63688.711	2	31844.356	4.192	.034
Error	121540.069	16	7596.254		
Total	243593.894	26			

Table C.19 Analysis of variance of Y in extraction step of *Hedyotis corymbosa* Lamk. infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	1876.937	2	938.468	25.147	.000
Time	215.405	2	107.703	2.886	.085
Temp * Time	126.315	4	31.579	.846	.516
Rep	21.983	2	10.991	.295	.749
Error	597.109	16	37.319		
Total	2837.748	26			

Table C.20 Analysis of variance of x in extraction step of *Hedyotis corymbosa* Lamk. infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	.005	2	.003	12.321	.001
Time	.001	2	.001	2.386	.124
Temp * Time	.002	4	.000	1.878	.163
Rep	.000	2	8.86E-005	.418	.665
Error	.003	16	.000		
Total	.011	26			

Table C.21 Analysis of variance of y in extraction step of *Hedyotis corymbosa* Lamk. infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	.000	2	5.84E-005	.055	.946
Time	.001	2	.001	.649	.536
Temp * Time	.006	4	.002	1.434	.268
Rep	.002	2	.001	1.052	.372
Error	.017	16	.001		
Total	.027	26			

Table C.22 Analysis of variance of total soluble solid in extraction step of *Hedyotis corymbosa* Lamk. infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	.112	2	.056	8.107	.004
Time	.014	2	.007	1.020	.383
Temp * Time	.048	4	.012	1.745	.189
Rep	.003	2	.001	.215	.809
Error	.110	16	.007		
Total	.287	26			

Table C.23 Analysis of variance of pH in extraction step of *Hedyotis corymbosa* Lamk. infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	.320	2	.160	50.224	.000
Time	.064	2	.032	9.975	.002
Temp * Time	.758	4	.189	59.404	.000
Rep	.026	2	.013	4.075	.037
Error	.051	16	.003		
Total	1.218	26			

Table C.24 Analysis of variance of antioxidant activity in extraction step of *Hedyotis corymbosa* Lamk. infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	847.410	2	423.705	142.719	.000
Time	2825.138	2	1412.569	475.803	.000
Temp * Time	1297.584	4	324.396	109.268	.000
Rep	16.479	2	8.239	2.775	.092
Error	47.501	16	2.969		
Total	5034.111	26			

Table C.25 Analysis of variance of ascorbic acid in extraction step of *Hedyotis corymbosa* Lamk. infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	2.070	2	1.035	2.843	.088
Time	1.196	2	.598	1.643	.224
Temp * Time	.187	4	.047	.128	.970
Rep	.131	2	.065	.180	.837
Error	5.824	16	.364		
Total	9.408	26			

Table C.26 Analysis of variance of total phenolic compounds in extraction step of *Hedyotis corymbosa* Lamk. infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	12354.706	2	6177.353	900403.684	.000
Time	27233.167	2	13616.584	1984737.198	.000
Temp * Time	257.484	4	64.371	9382.640	.000
Rep	.008	2	.004	.571	.576
Error	.110	16	.007		
Total	39845.474	26			

Table C.27 Analysis of variance of potassium in extraction step of *Hedyotis corymbosa* Lamk. infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	78194.434	2	39097.217	17.466	.000
Time	18106.733	2	9053.367	4.044	.038
Temp * Time	2948.087	4	737.022	.329	.854
Rep	18360.397	2	9180.198	4.101	.036
Error	35815.999	16	2238.500		
Total	153425.649	26			

Table C.28 Analysis of variance of Y in extraction step of *Orthosiphon aristatus* Miq infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	49.479	2	24.740	1.002	.389
Time	23.553	2	11.777	.477	.629
Temp * Time	165.012	4	41.253	1.672	.206
Rep	3.848	2	1.924	.078	.925
Error	394.872	16	24.680		
Total	636.765	26			

Table C.29 Analysis of variance of x in extraction step of *Orthosiphon aristatus* Miq infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	.002	2	.001	5.245	.018
Time	.002	2	.001	3.928	.041
Temp * Time	.001	4	.000	1.693	.201
Rep	3.10E-005	2	1.55E-005	.073	.930
Total	.009	26			

Table C.30 Analysis of variance of y in extraction step of *Orthosiphon aristatus* Miq infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	.002	2	.001	5.796	.013
Time	.001	2	.001	4.816	.023
Temp * Time	.001	4	.000	1.549	.236
Rep	1.82E-005	2	9.10E-006	.067	.936
Error	.002	16	.000		
Total	.006	26			

Table C.31 Analysis of variance of total soluble solid in extraction step of *Orthosiphon aristatus* Miq. infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	.009	2	.004	.727	.499
Time	.127	2	.063	10.364	.001
Temp * Time	.051	4	.013	2.091	.130
Rep	.002	2	.001	.182	.835
Error	.098	16	.006		
Total	.287	26			

Table C.32 Analysis of variance of pH in extraction step of *Orthosiphon aristatus* Miq. infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	.389	2	.194	51.072	.000
Time	.163	2	.081	21.403	.000
Temp * Time	.504	4	.126	33.111	.000
Rep	.010	2	.005	1.361	.284
Error	.061	16	.004		
Total	1.127	26			

Table C.33 Analysis of variance of antioxidant activity in extraction step of *Orthosiphon aristatus* Miq. infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	268.941	2	134.471	10.264	.001
Time	3703.516	2	1851.758	141.338	.000
Temp * Time	150.865	4	37.716	2.879	.057
Rep	21.819	2	10.909	.833	.453
Error	209.626	16	13.102		
Total	4354.767	26			

Table C.34 Analysis of variance of ascorbic acid in extraction step of *Orthosiphon aristatus* Miq. infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	11.379	2	5.689	23.808	.000
Time	1.085	2	.542	2.270	.136
Temp * Time	.216	4	.054	.226	.920
Rep	1.897	2	.949	3.970	.040
Error	3.824	16	.239		
Total	18.400	26			

Table C.35 Analysis of variance of total phenolic compounds in extraction step of *Orthosiphon aristatus* Miq. infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	548.828	2	274.414	66323.644	.000
Time	730.262	2	365.131	88249.139	.000
Temp * Time	160.660	4	40.165	9707.542	.000
Rep	.002	2	.001	.250	.782
Error	.066	16	.004		
Total	1439.818	26			

Table C.36 Analysis of variance of potassium in extraction step of *Orthosiphon aristatus* Miq. infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	7737.309	2	3868.655	1.814	.195
Time	13528.937	2	6764.468	3.173	.069
Temp * Time	2237.520	4	559.380	.262	.898
Rep	5718.396	2	2859.198	1.341	.289
Error	34114.013	16	2132.126		
Total	63336.175	26			

Table C.37 Analysis of variance of effect of sugar concentration on average score of liking of *Imperata cylindrical* (L.) P. Beauv. infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sugar	4.822	2	2.411	1.258	.292
Rep	71.822	29	2.477	1.292	.201
Error	111.178	58	1.917		
Total	187.822	89			

Table C.38 Analysis of variance of effect of sugar concentration on average score of liking of *Murdannia loriformia* (Hassk.) Rolla Rao et Kammathy infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sugar	7.489	2	3.744	1.560	.219
Rep	89.156	29	3.074	1.281	.209
Error	139.178	58	2.400		
Total	235.822	89			

Table C.39 Analysis of variance of effect of sugar concentration on average score of liking of *Hedyotis corymbosa* Lamk. infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sugar	169.089	2	84.544	27.823	.000
Rep	109.822	29	3.787	1.246	.235
Error	176.244	58	3.039		
Total	455.156	89			

Table C.40 Analysis of variance of effect of sugar concentration on average score of liking of *Orthosiphon aristatus* Miq infusion at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sugar	6.689	2	3.344	1.039	.360
Rep	68.322	29	2.356	.732	.819
Error	186.644	58	3.218		
Total	261.656	89			

Table C.41 Analysis of variance of bitterness of herbal infusions

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Herb	1010.763	3	336.921	141.982	.000
Rep	233.677	29	8.058	3.396	.000
Error	206.450	87	2.373		
Total	1450.890	119			

Table C.42 Analysis of variance of effect of *Imperata cylindrical* (L.) P. Beauv. infusion concentration on average score of liking of mixed herbal drink at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
I. cylindrical	42.300	3	14.100	7.610	.000
Rep	42.467	29	1.464	.790	.760
Error	161.200	87	1.853		
Total	245.967	119			

Table C.43 Analysis of variance of effect of sugar concentration on average score of liking of mixed herbal drink at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Sugar	90.825	3	30.275	53.291	.000
Rep	99.075	29	3.416	6.014	.000
Error	49.425	87	.568		
Total	239.325	119			

Table C.44 Analysis of variance of Y in sterilization process of sterilized mixed herbal drink at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	1.274	2	.637	2.566	.108
F_0	1005.019	2	502.510	2024.593	.000
Temp * F_0	10.467	4	2.617	10.543	.000
Rep	1.730	2	.865	3.484	.055
Error	3.971	16	.248		
Total	1022.461	26			

Table C.45 Analysis of variance of x in sterilization process of sterilized mixed herbal drink at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	.000	2	.000	645.941	.000
F_0	.002	2	.001	4236.699	.000
Temp * F_0	6.33E-005	4	1.58E-005	63.065	.000
Rep	1.61E-007	2	8.04E-008	.320	.730
Error	4.01E-006	16	2.51E-007		
Total	.003	26			

Table C.46 Analysis of variance of y in sterilization process of sterilized mixed herbal drink at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	.000	2	.000	1138.548	.000
F_0	.001	2	.001	5860.987	.000
Temp * F_0	.000	4	3.02E-005	327.037	.000
Rep	7.83E-007	2	3.91E-007	4.241	.033
Error	1.48E-006	16	9.23E-008		
Total	.001	26			

Table C.47 Analysis of variance of total soluble solid in sterilization process of sterilized mixed herbal drink at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	.020	2	.010	1.108	.354
F_0	.020	2	.010	1.108	.354
Temp * F_0	.013	4	.003	.369	.827
Rep	.029	2	.014	1.600	.233
Error	.144	16	.009		
Total	.227	26			

Table C.48 Analysis of variance of pH in sterilization process of sterilized mixed herbal drink at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	.000	2	.000	.337	.719
F_0	.001	2	.000	.571	.576
Temp * F_0	.001	4	.000	.736	.581
Rep	.000	2	.000	.384	.688
Error	.008	16	.000		
Total	.010	26			

Table C.49 Analysis of variance of antioxidant activity in sterilization process of sterilized mixed herbal drink at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	7.898	2	3.949	1.609	.231
F_0	240.328	2	120.164	48.948	.000
Temp * F_0	2.629	4	.657	.268	.894
Rep	1.305	2	.653	.266	.770
Error	39.279	16	2.455		
Total	291.439	26			

Table C.50 Analysis of variance of total phenolic compounds in sterilization process of sterilized mixed herbal drink at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	17.323	2	8.661	1.413	.272
F_0	2298.697	2	1149.349	187.461	.000
Temp * F_0	25.647	4	6.412	1.046	.415
Rep	8.350	2	4.175	.681	.520
Error	98.098	16	6.131		
Total	2448.116	26			

Table C.51 Analysis of variance of potassium in sterilization process of sterilized mixed herbal drink at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Temp	11.599	2	5.799	1.211	.324
F_0	22.899	2	11.450	2.390	.124
Temp * F_0	10.994	4	2.748	.574	.686
Rep	45.133	2	22.566	4.710	.025
Error	76.652	16	4.791		
Total	167.277	26			

Table C.52 Analysis of variance of Y of sterilized mixed herbal drink storage at 30 °C at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Week	412.069	17	24.239	384.610	.000
Rep	.561	2	.280	4.449	.019
Error	2.143	34	.063		
Total	414.772	53			

Table C.53 Analysis of variance of x of sterilized mixed herbal drink storage at 30 °C at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Week	.002	17	9.44E-005	1245.672	.000
Rep	1.24E-007	2	6.22E-008	.821	.448
Error	2.58E-006	34	7.58E-008		
Total	.002	53			

Table C.54 Analysis of variance of y of sterilized mixed herbal drink storage at 30 °C at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Week	.000	17	1.26E-005	186.509	.000
Rep	2.48E-008	2	1.24E-008	.184	.832
Error	2.29E-006	34	6.73E-008		
Total	.000	53			

Table C.55 Analysis of variance of total soluble solid of sterilized mixed herbal drink storage at 30 °C at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Week	.076	17	.004	1.015	.468
Rep	.009	2	.005	1.044	.363
Error	.151	34	.004		
Total	.236	53			

Table C.56 Analysis of variance of pH of sterilized mixed herbal drink storage at 30 °C at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Week	.001	17	8.33E-005	1.861	.060
Rep	.000	2	7.22E-005	1.613	.214
Error	.002	34	4.48E-005		
Total	.003	53			

Table C.57 Analysis of variance of antioxidant activity of sterilized mixed herbal drink storage at 30 °C at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Week	73.440	17	4.320	35.103	.000
Rep	.036	2	.018	.148	.863
Error	4.184	34	.123		
Total	77.660	53			

Table C.58 Analysis of variance of total phenolic compounds of sterilized mixed herbal drink storage at 30 °C at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Week	3605.576	17	212.093	9.205	.000
Rep	56.991	2	28.495	1.237	.303
Error	783.379	34	23.041		
Total	4445.945	53			

Table C.59 Analysis of variance of average score of liking of sterilized mixed herbal drink storage at 30 °C at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Week	582.592	17	34.270	65.686	.000
Rep	75.054	32	2.345	4.495	.000
Error	255.646	490	.522		
Total	917.665	539			

Table C.60 Analysis of variance of Y of sterilized mixed herbal drink storage at 45 °C at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Week	138.087	8	17.261	161.174	.000
Rep	.216	2	.108	1.008	.387
Error	1.714	16	.107		
Total	140.016	26			

Table C.61 Analysis of variance of x of sterilized mixed herbal drink storage at 45 °C at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Week	.000	8	3.11E-005	498.526	.000
Rep	9.41E-008	2	4.70E-008	.753	.487
Error	9.99E-007	16	6.25E-008		
Total	.000	26			

Table C.62 Analysis of variance of y of sterilized mixed herbal drink storage at 45 °C at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Week	8.05E-005	8	1.01E-005	133.738	.000
Rep	1.76E-007	2	8.78E-008	1.166	.337
Error	1.20E-006	16	7.53E-008		
Total	8.19E-005	26			

Table C.63 Analysis of variance of total soluble solid of sterilized mixed herbal drink storage at 45 °C at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Week	.045	8	.006	.718	.674
Rep	.001	2	.000	.047	.954
Error	.126	16	.008		
Total	.172	26			

Table C.64 Analysis of variance of pH of sterilized mixed herbal drink storage at 45 °C at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Week	.000	8	5.83E-005	1.200	.358
Rep	2.22E-005	2	1.11E-005	.229	.798
Error	.001	16	4.86E-005		
Total	.001	26			

Table C.65 Analysis of variance of antioxidant activity of sterilized mixed herbal drink storage at 45 °C at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Week	51.897	8	6.487	87.484	.000
Rep	.236	2	.118	1.590	.234
Error	1.186	16	.074		
Total	53.319	26			

Table C.66 Analysis of variance of total phenolic compounds of sterilized mixed herbal drink storage at 45 °C at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Week	860.213	8	107.527	1.877	.135
Rep	47.418	2	23.709	.414	.668
Error	916.819	16	57.301		
Total	1824.450	26			

Table C.67 Analysis of variance of average score of liking of sterilized mixed herbal drink storage at 45 °C at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Week	65.467	8	8.183	9.405	.000
Rep	71.467	29	2.464	2.832	.000
Error	201.867	232	.870		
Total	338.800	269			

Table C.68 Analysis of variance of Y of sterilized mixed herbal drink storage at 55 ^{o}C at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Week	789.557	8	98.695	441.892	.000
Rep	.390	2	.195	.873	.437
Error	3.574	16	.223		
Total	793.521	26			

Table C.69 Analysis of variance of x of sterilized mixed herbal drink storage at 55 ^{o}C at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Week	.000	8	6.09E-005	548.669	.000
Rep	2.23E-007	2	1.11E-007	1.004	.388
Error	1.78E-006	16	1.11E-007		
Total	.000	26			

Table C.70 Analysis of variance of y of sterilized mixed herbal drink storage at 55 ^{o}C at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Week	.000	8	2.86E-005	271.809	.000
Rep	1.65E-007	2	8.26E-008	.786	.473
Error	1.68E-006	16	1.05E-007		
Total	.000	26			

Table C.71 Analysis of variance of total soluble soilid of sterilized mixed herbal drink storage at 55 ^{o}C at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Week	.067	8	.008	1.111	.406
Rep	.000	2	.000	.000	1.000
Error	.120	16	.008		
Total	.187	26			

Table C.72 Analysis of variance of pH of sterilized mixed herbal drink storage at 55 °C at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Week	.001	8	7.59E-005	5.125	.003
Rep	2.96E-005	2	1.48E-005	1.000	.390
Error	.000	16	1.48E-005		
Total	.001	26			

Table C.73 Analysis of variance of antioxidant activity of sterilized mixed herbal drink storage at 55 °C at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Week	52.657	8	6.582	76.417	.000
Rep	.337	2	.169	1.959	.173
Error	1.378	16	.086		
Total	54.373	26			

Table C.74 Analysis of variance of total phenolic compounds of sterilized mixed herbal drink storage at 55 °C at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Week	1245.967	8	155.746	8.144	.000
Rep	235.340	2	117.670	6.153	.010
Error	305.967	16	19.123		
Total	1787.273	26			

Table C.75 Analysis of variance of average score of liking of sterilized mixed herbal drink storage at 55 °C at $p \leq 0.05$

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Week	278.163	8	34.770	48.642	.000
Rep	16.596	29	.572	.801	.758
Error	165.837	232	.715		
Total	460.596	269			

Biography

Bodin Techaratanakrai was born on July 25, 1984 in Bangkok. He graduated Bachelor's degree from Food Technology Department, Faculty of Science, Chulalongkorn University in 2006. Then he attended in postgraduated course in Food Technology Department, Faculty of Science, Chulalongkorn University in 2006 academic year.

Publication and Presentation

Bodin Techaratanakrai, Saiwarun Chaiwanichsiri, and Kalaya Laohasongkram. 2007. "Antioxidant activities of herbal infusions". Proceedings of the 33rd Congress on Science and Technology of Thailand. 18-20 October, 2007 at Walailak University, Nakhon Si Thammarat. Section G_G0028.