EXTRACTION AND DETERMINATION OF PROPERTIES OF NATURAL FOOD COLOR FROM BY-PRODUCT OF TURMERIC

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE
(FOOD AND NUTRITION FOR DEVELOPMENT)
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
2009

Thesis
entitled

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was submitted to the Faculty of Graduate Studies, Mahidol University
for the degree of Master of Science (Food and Nutrition for Development)
on
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ACKNOWLEDGEMENTS

I would like to express my deepest appreciation and gratitude to my advisor, Assist. Prof. Anadi Nitithamyong for her suggestion, helpful guidance, and encouragement throughout this study to make it successful. All her kindness will be long remembered in my mind.

I would like to thank with my sincere gratitude Assoc. Prof. Pongtorn Sungpuag, my co-advisor for his advice on the use of instrument, valuable comment and helpful technical instruction to perform all analysis throughout the study and thank to Dr. Warunee Varanyanond for her kindness in examining the thesis and suggestion who was a external examiner and the chair of my thesis defense.

The next person that I would like to thank is Asst. Prof. Sitima Jittinandana who gave precious comment and taught me to use the equipment in the Food Science and Technology laboratory. With her kindness, my thesis was finished and successful.

I would like to thank Mrs. Yupaporn Nakngamanong for HPLC technical suggestion and Miss Sirisuda Thanapatto for her kindness to teach me for antioxidant analysis.

I would like to thank the National Research Council of Thailand for the research grant in my research study, to Thai-China Flavours and Fragrances Industry Co., Ltd. for supporting the fresh ground turmeric by-product used as raw material in this study, to Rovithai Co., Ltd. for supporting dl-α-Tocopherol used as protective antioxidant agent, and also thank to International Flavors & Fragrances (Thailand) Ltd. for orange fragrance used in sensory evaluation samples.

My warmest thank to all graduate students, staff, and my friends at the Institute of Nutrition, Mahidol University who help me as panelists in my sensory evaluation for their kind cooperation and everything.

Finally, I would like to thank my family who always supports me. They have given me the spirit to do everything throughout of this study. Their warm love and care help me tremendously to reach this point. Thank you.

Wimalin Rimpeekool

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ABSTRACT

A by-product from the pharmaceutical industry, fresh ground turmeric, which is usually discarded from the processing facilities as fertilizer, was investigated to extract natural pigments for use as a food colorant. The objectives of this study were to prepare a natural food colorant from turmeric by-product, find an optimum condition for storage of the colorant and evaluate the consumer preference of the turmeric colorant added food products. Turmeric by-product was extracted with 95% ethanol and evaporated to a fifth fold concentration. The color extract was stored under three different storage conditions (4°C, room temperature and room temperature with dl-α-Tocopherol). Turmeric colorant contained at least three types of curcuminoid compounds (curcumin, demethoxycurcumin, bis-demethoxycurcumin). At day 0, the curcuminoid content was 9.9±1.2 mg/ml. Trolox equivalent antioxidant activity (TEAC) of DPPH and FRAP at day 0 was 3.04±0.19 and 2.94±0.17 mmol trolox/100 ml, respectively. The turmeric colorant showed different yellowish L*a*b* color parameters depending upon the type of solvent and pH level. Turmeric colorant was tested for its application in 8 different types of foods to determine the color value, appearance and consumer acceptance. As a result, the turmeric colorant could be used in various foods with good stability of color at high and low temperatures. Moreover, the scent of turmeric colorant seemed to make consumers accept the odor more when mixed in orange flavored products. Hence, the by-product of turmeric from the pharmaceutical industry could be used as source for preparing a natural colorant for application as a food coloring agent in general as well as health food products instead of using artificial colorings.

KEY WORDS: TURMERIC / COLORANT / BY-PRODUCT / CURCUMINOIDS / ANTIOXIDANT

108 pages

การสกัดและศึกษาสมบัติของสีผสมอาหารจากกากขมิ้นชัน

EXTRACTION AND DETERMINATION OF PROPERTIES OF NATURAL FOOD COLOR FROM BY-PRODUCT OF TURMERIC

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บทคัดย่อ

กากขมิ้นชันซึ่งเป็นส่วนที่เหลือทิ้งจากการสกัดสารสำคัญน้ำมันหอมระเหยในอุตสาหกรรมสมุนไพรถูกนำมาใช้ประโยชน์เป็นวัตถุดิบเพื่อผลิตสีผสมอาหารจากธรรมชาติ เพื่อเพิ่มมูลค่าให้กับกากขมิ้นที่แต่เดิมใช้ที่เพียงนำไปเป็นวัตถุดิบในการทำปุ๋ยเท่านั้น การวิจัยนี้มีจุดประสงค์ที่จะนำกากขมิ้นมาผลิตสีผสมอาหารและหาสภาวะที่เหมาะสมในการเก็บรักษา รวมไปถึงการทดสอบความพึงพอใจของผู้ชิมที่มีต่อผลิตภัณฑ์อาหารแต่งสีขมิ้นชัน ซึ่งในการวิจัย กากขมิ้นชันจะถูกนำมาสกัดด้วย 95% เอทานอล ด้วยอัตราส่วน 1 ต่อ 5 และถูกนำมาระเหยเพื่อทำให้มีความเข้มข้นขึ้น 5 เท่า ในการทดลองสารสกัดสีจะถูกนำมาบรรจุในขวดแก้วสีชาที่สภาวะอุณหภูมิ 4 องศาเซลเซียส, อุณหภูมิห้อง และ อุณหภูมิห้องที่เติมสารป้องกันเพิ่มเติม เพื่อค้นหาสภาวะเหมาะสมในการเก็บ สีที่สกัดได้ถูกนำมาตรวจสอบความเข้มข้นของสารสำคัญพบว่า มีความเข้มข้นของเคอร์คูมินอยด์ 9.9±1.2 มิลลิกรัมต่อมิลลิลิตรและตรวจพบว่ามีค่า TEAC จากการวิเคราะห์ DPPH และ FRAP เท่ากับ 3.04± 0.19 และ 2.94± 0.17 มิลลิโมลโทรลอกซ์ต่อ 100 มิลลิลิตร ตามลำดับ สีขมิ้นชันจะให้สีเหลืองที่แตกต่างกันแล้วแต่ตัวทำละลายและความเป็นกรด-ด่าง นอกจากนี้สีสกัดขมิ้นชันถูกนำมาประเมินประสาทสัมผัสด้วยอาหาร 8 ชนิด เพื่อศึกษาค่าสี, ลักษณะที่ปรากฏและความพึงพอใจที่มีต่ออาหารแต่งสีขมิ้นชัน ผลที่ได้พบว่า สีขมิ้นชันที่ได้มีความคงตัวของสีทนต่ออุณหภูมิในการประกอบอาหารทั้งร้อนและเย็น แม้ว่าจะมีกลิ่นของน้ำมันหอมระเหยของขมิ้นหลงเหลืออยู่ แต่สามารถเสริมกลิ่นของอาหารที่เติมกลิ่นส้มได้ สรุปได้ว่า กากขมิ้นชันที่ได้จากอุตสาหกรรมสมุนไพรสามารถนำมาเป็นวัตถุดิบในการผลิตสีธรรมชาติ เพื่อใช้ในผลิตภัณฑ์อาหารทั่วไปและอาหารสุขภาพทดแทนสีสังเคราะห์ได้

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LIST OF ABBREVIATIONS

HPLC	high-performance liquid chromatography
GC-MS	gas chromatography mass spectrometry
DPPH	DPPH free radical scavenging method
FRAP	ferric-reducing antioxidant power assay
CIE	the Commission Internationale de l'Eclairage
FD&C Act	the Federal Food, Drug, and Cosmetic Act
FDA	Food & Drug Administration
CFR	Code of Federal Regulations
GRAS	generally recognized as safe
C.I.	color index
CAS	Chemical Abstract Service
SCF	Scientific Committee for Food
JECFA	the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives
CC	curcumin
DMC	demethoxycurcumin
BDMC	bisdemethoxycurcumin
g	gram
L	liter
mg	milligram
µg	microgram
ml	milliliter
mmol	millimole
cm	centrimeter
°C	degree Celsius

CHAPTER I
INTRODUCTION

In recent years, there has been a growing interest among food manufacturers and consumers regarding the application of natural food colors in food products. The trend is to use natural colors as an alternative to chemical food colors to enhance safety and reliability in the products. Many studies evaluated the properties of natural colorant and their applications to use instead of synthetic colorants in food and beverage (1-5). The main reasons behind this trend include an increase in consumer awareness on health as well as research results that indicate a possibility of chemical color to cause allergy. Although the conclusions of chemical color allergy studies are still unclear, a natural alternative seems to be the key for the future (6, 7).

Several plants and certain animals have been used or studied as sources of food colors. One group of interesting materials is by-products from food or related industries. In the past, by-products from food and pharmaceutical industries became a major disposal problem. They are usually discarded from the processing facilities as fertilizer. Preparation of natural food colors from these by-products may be one way of adding value and improving utilization efficiency. The example of uses of by-product from herb production for food was shown in published studies by Mielnik MB (8) Negi PS *et al.*(9).

Turmeric or *Curcuma longa* L. is commonly used in many ways. It is a popular spice used for food coloring and flavoring as well as treatment of several diseases in traditional medicine (10, 11). Turmeric has widely been studied for its properties such as antioxidant, anti-inflammatory, antibacterial, anti-tumor, anti-angiogenic, wound healing and anti-cancer (9, 12, 13).

Therefore, a by-product of turmeric production from pharmaceutical industry should be investigated for use as a raw material for color extraction. Fat-soluble yellow pigments, called curcuminoids, can be extracted from this by-product. Curcuminoids refer to a group of phenolic compounds found in 3-5% of turmeric.

Three major curcuminoids are curcumin, demethoxycurcumin (curcumin II) and bisdemethoxycurcumin (curcumin III) (14, 15). Curcumin is a permitted yellow color like tartrazine, an artificial azo dye, commonly used in human food and pharmaceutical products (7, 16). In addition; curcumin has its antioxidant activity comparable to vitamins C and E. It is a potent scavenger of many reactive oxygen species including superoxide anion radicals, hydroxyl radicals and nitrogen dioxide radicals (17). Because of its antioxidant activity, curcumin has been shown to exhibit anti-carcinogenic and anti-mutagenic properties (13). Curcumin can block the formation of maillard reaction products and their mutagenic activity (18). Many researches suggested that a dietary supplementation with curcumin may be beneficial because it has potential in the treatment of multiple diseases (13).

In the study of natural color extraction, curcuminoids are extracted from turmeric by-product with solvents. Many methods can be used to analyze the active components of turmeric extraction such as high-performance liquid chromatography (HPLC) and gas chromatography mass spectrometry (GC-MS) (19-22). However, previous researches suggested that bioactive components could be lost under preparation and processing conditions. Curcuminoid pigments in aqueous solutions were degraded by alkaline, with maximum degradation at pH 10.2 and tended to decline at higher pH (23, 24). Heat processing of turmeric caused 27-53% loss of curcumin active principle, with the maximum loss occurred in pressure cooking for 10 minutes (25).

One study compared the stability of natural turmeric color to synthetic color like tartrazine for color, moisture content and pigment retention (26). After 10 weeks of storage, curcumin degradation showed a faster rate than that of tartrazine. Nevertheless, turmeric could still be used as an alternative for tartrazine. Another study also suggested that natural colorant (curcumin) could replace artificial colorants found in a product like commercial jelly (27).

Curcumin, as antioxidants, have been tested both *in vivo* and *in vitro*. Many researches determined the antioxidant capacity using various means; such as, tumor development, DPPH free radical scavenging method, lipid peroxidation method, ferric-reducing antioxidant power assay (FRAP) (12, 14, 28, 29). These tests are now available for determination of antioxidant activity in curcumin.

In this study, major pigments were extracted from turmeric by-product from pharmaceutical industry using alcoholic extraction method to produce a natural colorant (30). Factors that can affect the change of active components in the colors such as temperature and protective agent were experimented to find an optimum storage condition. Curcuminoid concentration, CIE L*a*b* color parameters, and antioxidant capacity with DPPH and FRAP assay were determined. Application of the color extract in various food products was tested. Sensory evaluation of the products was performed to determine consumer acceptance.

CHAPTER II
OBJECTIVES

2.1 General objective

To prepare a natural food colorant from by-product of turmeric (*Curcuma longa*) from pharmaceutical industry and to test its application in different types of food products

2.2 Special objectives

1. To prepare a natural food colorant from ground fresh turmeric by-product
2. To study the stability of the natural food colorant under different conditions to find an optimum storage condition for the colorant
3. To determine the curcuminoids content of turmeric colorant throughout study
4. To determine the antioxidant properties of the colorant by using DPPH and FRAP assays
5. To determine the CIE L*a*b* color parameters of turmeric colorant in various conditions
6. To apply the colorant in different types of food products
7. To evaluate the consumer acceptability of the turmeric colorant added food products

2.3 Expected outcomes

1. Turmeric by-product from pharmaceutical industry could be used as a source of turmeric colorant
2. Curcuminoid concentration and antioxidant activity of the extracted turmeric colorant were determined
3. Application of turmeric colorant in food products was studied.

CHAPTER III
LITERATURE REVIEW

3.1 Food colorant

The color of food product can be changed to colorless before it reaches the consumers because of color degradation during processing steps and transportation of product. The use of colorant is necessary to restore the original food color appearance and/or to obtain color uniformity to make a good visual characteristic in food quality for consumer acceptance (31).

3.1.1 Definition

Colorant is any material (dye, pigment or any substance) that is used to add in food or drink to change its color and improve the appearance. Colorant can be either natural or synthetic colorant. It is added to food product in specific processing step to maintain desired color of product and increase color intensity to make a good quality product and to gain consumer acceptance (32). Colorant can used to compensate the loss of color and restore a natural color of food that can be lost by exposure to light, air, temperature, moisture, and storage condition (33).

3.1.2 Classification of food colorant

Food colorants have been classified by different systems. Today, the most common classifications are based on their origin and legislation.

Food colorant based on the origin of colorant can be classified into 3 types (34) as follows.

a. Natural: colorants are synthesized, accumulated or excreted from a living organism.

b. Nature-identical: colorants are produced by a chemical synthesis to have a similar chemical structure to colorants found in nature (35).

c. Synthetic: colorants are chemically synthesized and do not occur in nature.

Some colors such as those produced from maillard reaction during baking cannot be clearly classified into “natural” or “synthetic” type (34).

Classification of colorants based on chemical structure is still not clear. Most experts agree to classify natural colors into 3 chemical classes; isoprenoid derivatives, tetrapyrrole derivatives and benzopyran derivatives while some experts also classify the artifacts (melanoidins and caramels) in the fourth group (34).

Furthermore, colorants can be classified based on other basis such as solubility, applications, global chemical characteristic, structural characteristic and legislation (36).

3.1.3 International legislations of food colorant

3.1.3.1 United States legislations

In the Federal Food, Drug, and Cosmetic Act (FD&C Act), colorant is a part of food additives. United States legislation provides the certified color additive for use in food, drugs, cosmetics and some medical devices. The US Food & Drug Administration (USFDA) establishes the regulations for food additives in Title 21 of Code of Federal Regulations (CFR) in the part 70 to 82 that cover different parts concerning food additives such as definition of a color additive and certification requirement. Furthermore, some coloring additives are generally recognized as safe (GRAS) by the USFDA and do not require certification (36, 37).

Under the US food legislation, each color has its color index name (C.I. name), color index number (C.I. No.) and Chemical Abstract Service registry code numbers (CAS registry number) (38). *The color index name* of colorant is derived from application class, the color or hue of colorant and a sequential number. Color index name is created to use instead of trade name. For example; C.I. name of tartrazine is Food Yellow 4. *The color index number* is comprised of five digits assigned by the manufacturer. Although the colorants from different company have the same C.I. No., the purity and chemical constituent will be different. For example; C.I. No. of tartrazine is 19140. *CAS registry number*, a group of number, is set by Chemical Abstract Service. CAS number has three parts of number separated

with hyphen. The first part consists of 2-6 digits, the second part consists of two digits, and the third part consists of a single digit. For example; CAS number of curcumin is 458-37-7.

Food colorant has been classified based on legislation into 2 types (31, 34).

a. Certifiable colors: Synthetic, organic colorants, dyes, and lakes that can be used after obtained previous certification of USFDA from color manufacturer, analysis acknowledgment and a assignment of a certification number. Colorants which are produced from anthropogenic synthetics are contained in this type (36).

"FD&C" is preceding the colorant name that a colorant is certifiable for foods, drugs, or cosmetics. For example; FD&C Yellow No. 5

"D&C" is preceding the colorant name that it is approved for use only in drugs and cosmetics. For example; D&C Red No. 6

"Ext. D&C" is preceding the colorant name that it is approved for use in externally applied drugs and cosmetics. For example; Ext. D&C Violet No.2

b. Exempt colors (Exempt from certification): Natural organic or inorganic colors that do not require certification but purity specifications must be followed by both the manufacturing and consuming companies. They are permitted for use under the CFR. Exempt colors include 26 natural colorants, derived or originating from natural sources (vegetable, animal or mineral) and 17 synthetic colorants (31, 36). The names of colorant are based on common name, chemical structure and origin such as caramel, titanium dioxide and curcumin, respectively.

3.1.3.2 European Union legislations

Food additives directives are controlled by EU legislation. The EU directives are based on the recommendations of the Scientific Committee for Food (SCF), the Codex Alimentarius Commission, and the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA)(36).

Approved dyes are also defined as food additives and thus carry E numbers (E=EEC number of food additives). The E-number of color additives is in the range of 100 to 199. A pigment derived from extraction has a differential

specific as the chemical synthesis thus its may be a differentiated E number. In each states of EU, domestic regulations may be different depending on various additives. The community regulation on food additives comprise of many directives such as Council Directive 89/107/EEC and European Parliament and Council Directive 94/36/EC (36, 37).

Council Directive 89/107/EEC amended by Directive 94/34/EC is a framework directive for developing individual additive directives. This directive contains a food additive definition, categories of food additives and general criteria for use of food additives (36).

European Parliament and Council Directive 94/36/EC: on colors for food stuff in European Union contains a list of permitted colorant, a list of foodstuff which only certain permitted color can be used or limited list of colorants used or not allowed to use any colorants (36).

Commercial synthetic colorants with yellow-orange color

Tartrazine: The synonyms of tartrazine are known as CI Food Yellow 4, FD&C Yellow No. 5, CI (1975) No. 19140, E No. 102 The chemical name is trisodium 5-hydroxy-1-(4-sulfonatophenyl)-4-(4-sulfonatophenylazo)-H-pyrazole-3-carboxylate. Its CAS number is 1934-21-0. Formula weight is 534.37. Tartrazine is a light orange powder or granules, soluble in water, sparingly soluble in ethanol. Tartrazine can be dissolved in water to give a golden yellow color. In water, the maximum absorption is at 426 nm, $E_{1cm}^{1\%} = 530$. Tartrazine has good pH stability and not fading after one week at pH 3-8. This colorant also has good heat and light stability but poor stability in the presence of ascorbic acid. Tartrazine can be safely applied in foods. JECFA established the use of tartrazine up to 300mg/ml in various foods (Figure 1) (36, 39).

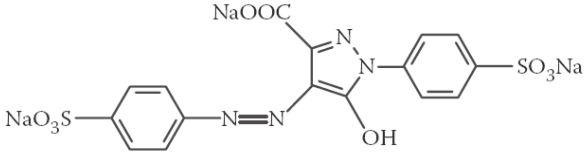

Figure 1 Chemical structure of tartrazine (36)

Sunset yellow FCF : The synonyms of sunset yellow are known as CI Food Yellow 3, FD&C Yellow No. 6, Crelborange S, CI (1975) No. 15985, E No. 110 The chemical name is disodium 6-hydroxy-5-(4-sulfonatophenylazo)-2-naphthalene-sulfonate. The CAS number is 2783-94-0. Formula weight is 452.37. Sunset yellow is a brown-orange powder or granules, soluble in water to give yellow-orange color, sparingly soluble in ethanol. In water, at pH 7, the maximum absorption is at 485 nm, $E_{1cm}^{1\%} = 555$. Sunset yellow has a good stable shade of color at pH 3-8 after one week. It is less stable in heat and light than tartrazine and also poorly stable in the presence of ascorbic acid. Sunset yellow can be safely used in foods. JECFA established the use of sunset yellow up to 300mg/ml in various foods (Figure 2) (36, 39).

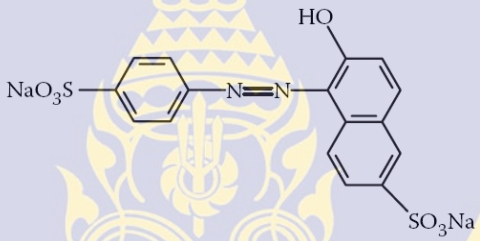

Figure 2 Chemical structure of sunset yellow (36)

3.2 Natural colorant

Natural colorants seem to be a preferable alternative for consumers instead of synthetic colorant to enhance safety and health. Although a natural colorant is more complex and expensive to produce than synthetic colorant, food producer tends to accept a significant production cost for adding natural food additive and color.

3.2.1 Definition

Natural colorant is a color that produces from a living organism such as animal, plant, fungi, and microorganism. Natural colorant is usually extracted from the raw material and unprocessed food and agricultural product, particularly vegetable, fruit, seed, and root. The best known natural colorants in application are anthocyanins, carotenoids, and chlorophylls (31, 36).

Anthocyanins: Anthocyanins are flavonoids commonly found in nature such as in red onion, fig, strawberry, mango, and grape. The extract tends to be dark black or purple color. Anthocyanins are used as coloring compounds in foodstuff such as dairy product, beverage, sugar product, confectionery, coating, and dessert (40).

Chlorophylls: Chlorophylls, green pigments, are the most abundant pigment in nature. Chlorophylls can be found in all plants, ferns, mosses, algae and some photosynthetic bacteria. This pigment is a food colorant that has a wide range of application in food industries such as dairy product, soft drink, confectionary and dessert (33, 40).

Carotenoids: Carotenoids are red, orange and yellow pigments. Carotenoids can be isolated from red-yellow plants and some can come from animal sources. The commonly used carotenoids in food are paprika, saffron, carrot extracts, lycopene and ß-carotene. This pigment is used as a colorant in fermented vegetable product, pasta, noodles, cereal product, coating and seasonings.(33, 40)

Curcumin: Curcumin is an orange-yellow pigment in color extraction from rhizome of turmeric. This pigment is commonly used in many forms such as curcumin, oleoresin, and turmeric powder (41). The information of curcumin is shown in Section 3.4

3.3 Turmeric

Turmeric (or Kamin chan in Thai language) is a popular spice that has been widely used in many countries. The origin of turmeric is not clear but probably in Southeast Asia (41). Although India is the biggest turmeric producer and exporter in the world, many studies found that turmeric from Thailand has high quality in amount of essential oils and curcuminoids (42). Thai people have known the turmeric for centuries as food colorant, preservative, flavoring and traditional medicine purpose.

3.3.1 Name and synonyms

Turmeric is a name that originated from Medieval Latin in name *terra merita* which becomes *terre merite* in French, meaning deserved earth or meritorious earth, a name by which powdered turmeric was known in commerce (41, 43).

The scientific name of turmeric is *Curcuma longa* L. Curcuma is a Latin name derived from Kourkoum, a word of Arabic origin, meaning saffron, and also the name is established by Linnaeus in 1753 for this kind of monocotyledonous herb in India (44).

In Thailand, the common name of turmeric is Ka-min. Other names in different provinces in Thailand such as Kamin kang, Kamin yonk, Kamin hou, Kimin, Min, Tayo and Sayo (42). Other common names of turmeric are different depending on the languages such as Indian saffron in English, chiang husang in Chinese as shown in Table 1 (15, 41).

3.3.2 Taxonomy of turmeric

Turmeric (*Curcuma longa* L.) is a member in a genus *Curcuma*, belonging to the family Zingiberaceae that contains about 110 species. This genus is subdivided into 3 sections; exantha, mesantha, and hitcheniopsis. Turmeric is a member of section exantha that also has other economically important species such as *C. anguistifolia* Roxb. (Indian arrow root) (41, 45). The systematic classification of turmeric is shown in Table 2.

Table 1 Local names and synonyms of turmeric (15, 41, 46)

Local name	
Thailand	Kamin, Kamin kang, Kamin yonk, Kamin hou, Kimin, Min, Tayo and Sayo
English	Turmeric, Indian saffron
French	Curcuma, Saffron de India, sochet des Indes, souchet, souchet long, souchet odorant, teri-merit
Japanese	Ukon, Tamerikku
Chinese	Chiang husang, Kiang husang, Yu chin, Yu jin, Wohng geung, Geung wohng, Wat gam, Huang jiang, Jiang huang, Yu jin, Yu jin xiang gen
Italian	*Curcuma*
Sanskrit	Ameshta, bahula, bhadra, dhirgharaja, gandaplashika, gauri, haldi, haridra, hemaragini, hridvilasini, jayanti, jwarantika, kanchani, krimighna, kshamada, kshapa, mangalaprada, mehagni, nisha, nishakhya, pavitra, pinga, pita, pitika, rabhangavasa, ranjani, ratrimanika, shifa, shobhana, shyama, soughagouhaya, suvarnavarna, tamasini, umavara, vauragi, varavarnini, varnadatri, varnini, vishagni, yohitapriya, yuvati
Synonym	*Curcuma domestica* Valeton., *C. rotunda* L., *C. xanthorrhiza* Naves, *Amomum curcuma* Jacq.

Table 2 The systematic classification (taxonomy) of turmeric (47).

Kingdom	Plantae - Plants
Subkingdom	Tracheobionta - Vascular plants
Superdivision	Spermatophyta - Seed plants
Division	Magnoliophyta - Flowering plants
Class	Monocotyledoneae - Monocotyledons
Subclass	Zingiberidae
Order	Zingiberales
Family	Zingiberaceae - Ginger family
Genus	*Curcuma* L. - curcuma
Species	*Curcuma longa* L. - common turmeric

3.3.3 Morphological description of plant

Turmeric plant has two major parts, leaf shoot and rhizomes. Leaf shoots consist of a group of green leaves surrounded by bladeless sheathes. The leaf sheaths may form 2-3 pseudostems per plant. The height of leaf shoots is about 1-2 meter (Figure 3). Turmeric flower is pale yellow color. The seed rhizome (planting unit) grown under the ground consists of two parts either bulb or finger. A central pear-shape bulb of turmeric rhizome is called “mother rhizome”, the lateral of rhizome has small axillary branches known as the “fingers”. The first order fingers that develop from the lower node of main axis are called “primary fingers” (Figure 4). The primary fingers can develop to produce the secondary and tertiary branches. Turmeric finger usually has brownish yellow ringed and scaly skin but inside is bright yellow orange color (Figure 5) (41, 42).

Figure 3 Turmeric plant (*Curcuma longa* L.) (41)

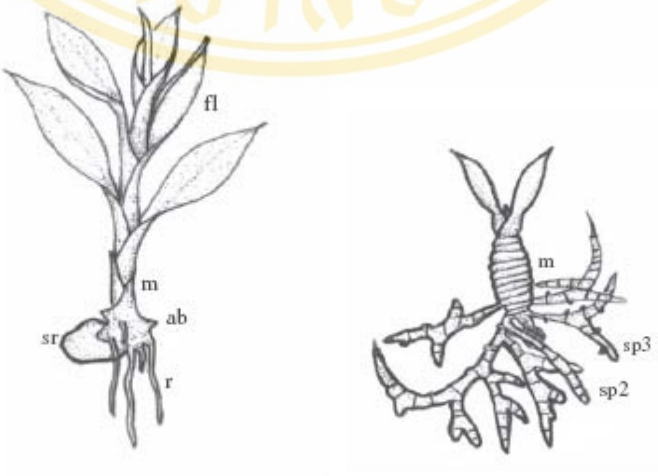

Figure 4 The parts of turmeric plant; axillary bud (ab), foliage leaf (fl), main axis (m), root (r), seed rhizome (sr), secondary branches (sp2), tertiary branches (sp3) (41).

Figure 5 Turmeric rhizome

3.3.4 Cultivation and harvesting season

Turmeric is grown wildly and planted in tropical countries such as India, Thailand, China, Indonesia, Laos, Cambodia and Vietnam. In Thailand, turmeric is suitable to cultivate in rainy seasons from May to July. Harvesting is performed in winter when the color of leaf shoots change to brown-yellow color in January to March. In India, turmeric is always planted in the end of April to August and harvested in December to March in various states dependent on the variety, planting material and climate condition (33, 42).

The yield of turmeric rhizomes depends on various factors such as species, nutrition, environment factor, planting material and method, and harvesting method. Turmeric is planted by the flat bed and the ridges and furrow methods. This plant grows well on loose and friable, well-drained, loamy or alluvial soils with pH range of 4.3 to 7.5. An optimum temperature for the growing of turmeric rhizome buds is in a range of 25-35°C. In open ground exposed to the sun, turmeric can produce a larger and better rhizome than in the shade. Irrigation is not required for turmeric crop where rainfall is bimodal and ample. The harvesting method begins with removing vegetative parts (leaves and stem) and digging by manual labor. About one fifth of harvested turmeric rhizomes is retained as a seed material for the next cultivation (41).

3.3.5 Nutritional composition of turmeric

Turmeric contains carbohydrate, fat, protein and other nutrients especially minerals as shown in Table 3. However, the nutrition composition may vary in other studies depending on species, cultivation and harvesting method.

Table 3 The nutritional composition of turmeric per 100 g. (41)

Composition	USDA Handbook	ASTA
Water (g)	11.36	6.0
Food energy (kcal)	354	390
Protein (g)	7.83	8.5
Fat (g)	9.88	8.9
Carbohydrates (g)	64.93	69.9
Ash (g)	6.02	6.8
Calcium (g)	0.182	0.2
Phosphorous (mg)	268	260
Sodium (mg)	38	10
Potassium (mg)	2525	2500
Iron (mg)	41.42	47.5
Thiamine (mg)	0.152	0.090
Riboflavin (mg)	0.233	0.190
Niacin (mg)	5.140	4.8
Ascorbic acid (mg)	25.85	50

3.3.6 Chemical constituents in turmeric

Fresh turmeric consists of 25-30% starch, 2-7% essential oil and 3-5% pigment (48). Many phytochemicals found in turmeric are identified as various types of secondary metabolites including diphenylheptanoids, monoterpenes and sesquiterpenes. Curcuminoids are a group of phenolic compounds that are predominantly found in turmeric. Their content in different varieties varies from 2-8% (49).

3.3.7 Traditional use of turmeric

Turmeric rhizomes are used in household remedy. Turmeric has been used as a stomachic, tonic, blood purifier and skin diseases prevention and treatment. Turmeric (powder form) used as medicine against biliary disorders, anorexia, coryza, cough, diabetic wounds, hepatic disorder, rheumatism and sinusitis (49). For food purpose, turmeric is used as an ingredient of mustard paste, curry powder and also used for vegetable and meat dishes and soup-like dishes in Asian. Turmeric powder is a major ingredient (40-50%) in curry powder (43). In Thailand, Turmeric is commonly used in yellow curries, curried rice, stew and meat dishes (50). Moreover, turmeric is used as a dye in coloring cotton fabrics and in calico printing (41).

3.3.8 Biological and medicinal properties of turmeric

Negi PS *et al.* (9) found turmeric oil from mother liquor that gained from oleoresin, a by-product from curcumin manufacture has an antibacterial activity against *Bacillus cereus, Bacillus coagulans, Bacillus subtilis, Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa.*

Ak T *et al.* (29) published that curcumin has an antioxidant activity to inhibit lipid peroxidation of linoleic acid emulsion. Moreover, curcumin is effective to scavenge DPPH radical, ABTS radical, DMPD radical, superoxide anion radical and hydrogen peroxide. It also has ferric ions (Fe3+) reducing power and ferrous ions (Fe2+) chelating activity. Inhibition of lipid peroxidation activity of curcumin demonstrated positive result in other studies such as that of Ruby AJ *et al.* (12). They also found that curcuminoids exhibited a potential for use as antitumor promoter.

Many researches showed the biological activity of curcumin as an antioxidant, anti-inflammatory, antitumor, antimicrobial and anticarcinogenic activities (15, 51-53). Moreover, curcumin has been reported to act as a cholesterol lowering agent by increasing conversion of cholesterol into bile, and increase mucin in stomach (45).

3.4 Curcuminiods

Three major active non-volatile compounds belong to the curcuminoid family; curcumin (CC), demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC). Curcumin, the major pigment of turmeric (about 77% of total curcuminoids), is a yellow-orange crystalline form and identified as 1,6-heptadiene-3,5-dione-1,7-bis(4-hydroxy-3-methoxyphenyl)-(1E,6E) or diferuloylmethane as shown in Figure 6. It contains two ferulic acid molecules linked via a methylene bridge at the C atoms of the carboxyl groups. Curcumin is poorly soluble in water and ether but soluble in various organic solvents such as ethanol, dimethylsulfoxide, and acetone. The melting point of curcumin is 183°C. Molecular formula is $C_{21}H_{20}O_6$ and molecular weight 368.37 g/mol. The maximum absorption of curcumin occurs in the range of 415-430 nanometers depending on a solvent (15, 36, 49). Curcumin has antioxidant activity comparable to vitamin C and E. Many studies reported its scavenging ability to many reactive oxygen species including superoxide radicals, hydroxyl radicals and nitrogen dioxide radicals (49).

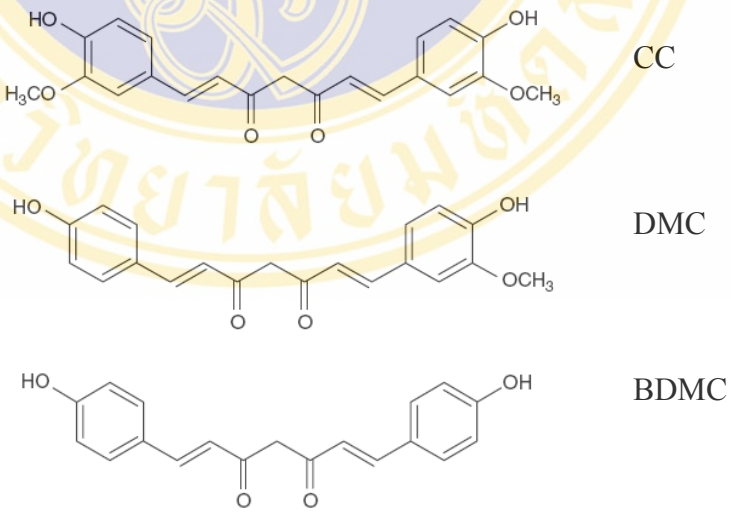

Figure 6 Structure of curcumin (CC) , demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) (43)

Demethoxycurcumin or 4-hydroxycinnamoyl-(4-hydroxy-3-methoxycinnamoyl) methane with one methoxy group has an orange-yellow color.

The melting point of demethoxycurcumin is 168 °C The maximum absorption of demethoxycurcumin measured at 424 nanometer when dissolved in alcohol (41).

Bisdemethoxycurcumin or bis-(4-hydroxy cinnamoyl) methane without methoxy group has a yellow color. The melting point of bisdemethoxycurcumin is 224 °C The maximum absorption of bisdemethoxycurcumin measured at 419 nanometer when dissolved in alcohol (41).

3.4.1 Antioxidant activities of curcuminoids

Curcumin commonly exists in an equilibrium form between the diketo and keto-enol forms by intramolecular H-bonding as appears in Figure 7. At pH 3–7, diketo form of curcumin (non-ionized curcumin) is predominating to act as an extraordinarily potent H-atom donor better than ionized curcumin. In keto form of curcumin, the heptadienone linkage between the two methoxyphenol rings contains a highly activated carbon atom, so the C–H carbon bonds on this carbon are very weak due to delocalization of the unpaired electron on the adjacent oxygens (52, 54, 55).

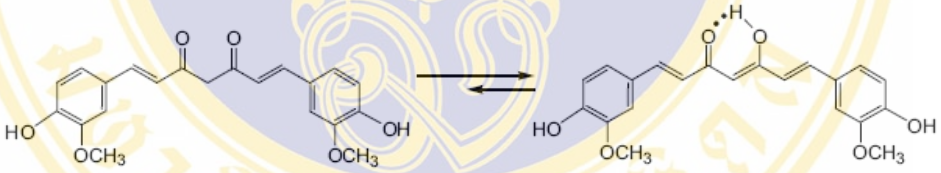

Figure 7 Equilibrium symmetrical form of curcumin: noionized curcumin (left) and noionized curcumin (right) (55)

Curcuminoids have been shown to be a free radical scavenger. Mechanisms of the radical trapping reactions of curcumin are shown in Figure 8. Curcumin, a stable form with two phenoxy (A), can trap a proton from any reagent such as acidic compound or any free radical. Then direct abstraction of phenolic hydrogen occurs through initial ionization from the central methylene. The electron is transferred to form carbon centered radical that can isomerize to gain the last form (B) in mechanisms as shown in Figure 8 (55).

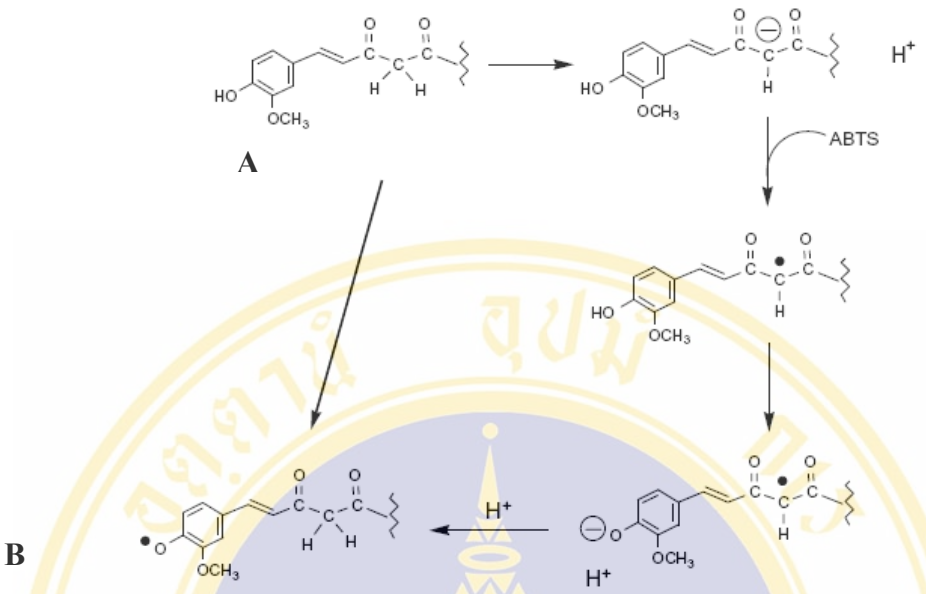

Figure 8 Mechanisms of the radical trapping reactions of curcumin (55)

3.4.2 Stability of curcuminiods

Curcumin is highly stable in acidic solution but poorly stable at neutral and alkaline solution. Curcumin has a bright yellow hue color at pH 2.5-7 and changes to red hue at above pH 7 (41). In a phosphate buffer at pH 7.2 within 30 minutes, large amount of curcumin may be degraded to four compounds that consist of trans-6-(4'-hydroxy-3'-methoxyphenyl)-2,4-dioxo-5-hexenal (major degradation product), vanillin, ferulic acid, and feruloyl methane (Figure 9) (24).

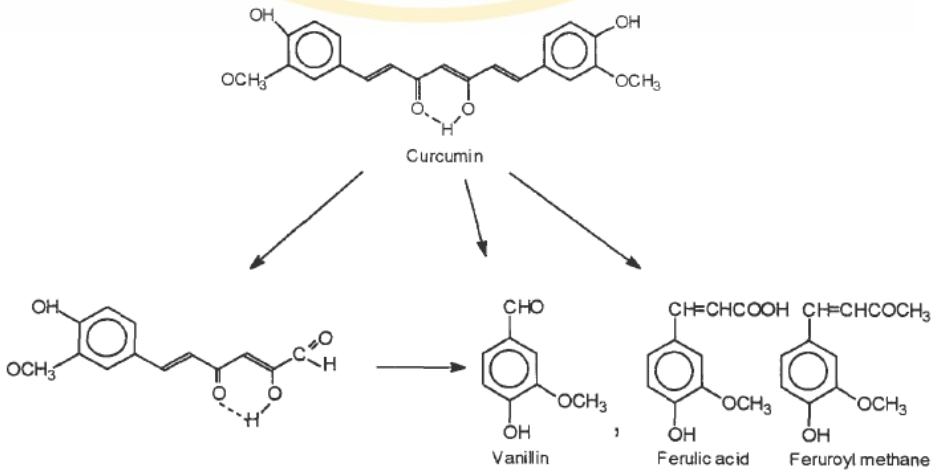

Figure 9 Chemical structures of degraded products of curcumin in 0.1 M phosphate buffer, pH 7.2 at 37°C (24).

On the other hand, Masuda T *et al.* proposed four products from radical reaction of curcumin consisting of curcumin and three radical products (two fragmented compounds from curcumin and one of which was structurally elucidated to form a dimer) (Figure 10) (56).

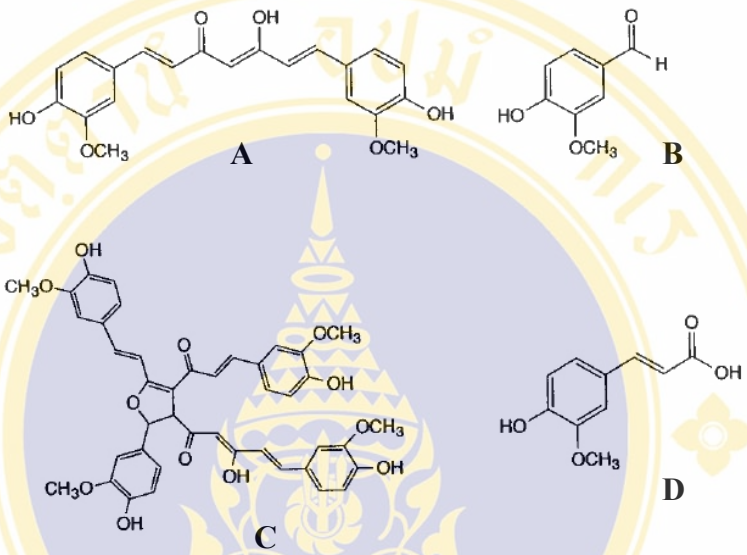

Figure 10 Chemical structures of three radical reaction products; curcumin (A) and three radical products (B-D) (56)

In acidic and neutral aqueous solution (pH 3-7), curcumin is a diketo form to act as an H-atom donor. In contrast, the keto-enol form of the heptadienone chain predominates and curcumin acts mainly as an electron donor at above pH 8 (52).

In brine, curcumin is more stable than both demethoxycurcumin and bisdemethoxycurcumin. The half-lives of curcuminoids range from 8.6-10.4 h in air atmosphere and 9.0-12.0 h in nitrogen atmosphere (31).

Curcumin is also sensitive to light and heating. Degradation can be prevented by protection from sunlight and addition of protective agents such as ascorbate or N-acetyl-cysteine (36, 52).

3.4.3 Extraction of curcuminoids

Curcuminoids can be extracted from turmeric by many methods that use different solvents such as alcohol extraction, partition separation with various solvents, soxhlet apparatus, precipitation with solvent, cold percolation extraction method, isolation with soap solution, supercritical carbon dioxide (CO_2) extraction and other methods (44). The commonly used solvents to extract oil fraction of turmeric are such as heptane, acetone, alcohol, and ethylene dichloride. The yield of curcuminoid extraction is in the range of 10-20% (31).

Jain V *et al.* used solvent extraction method by percolation with light petroleum ether at 60–80°C before removing solvent under vacuum below 50°C to keep oily residue. The curcuminoid content in oily residue using HPLC determination was found in the range of 0.32-0.55% (57). Several studies used non-edible solvents to extract oily parts. Conversely, Gilda S *et al.* used food-grade polyglycolized glycerides to extract water-soluble extract that contained lipophilic curcuminoids instead of using different non-edible organic solvents. Gelucire 44/14, an amphiphilic vehicles solvent, gave the maximum concentration of curcuminoids (58).

Began G *et al*. reported the optimization to extract oil fraction in supercritical CO_2 at an optimal pressure of 22.5 MPa. The rise of extract temperature led to decrease in oil yield while increasing flow rate gave an increase in oil yield (59). Chang L-H *et al.* used supercritical CO_2 to extract 75 g of 0.42mm turmeric powder at 60°C, 300 bar for 2.5 h and obtained 6.98% turmeric oil (60).

Steam distillation process with variable autoclave pressure and distillation time has been studied by Manzan ACCM *et al*. (61). The results showed the highest yield of essential oil (0.46%) and curcuminoids (0.16%) was obtained at a pressure of $1.0x10^5$ Pa for 2 h. On the other hand, extraction using volatile solvents at 40°C, 6 h provided the best yield of essential oil (5.49%) while the best yield of pigment (7.98%) was obtained under the same conditions, except for the temperature (30°C).

Braga MEM *et al.* compared the yield of curcuminoid content obtained using various techniques i.e. hydrodistillation, low pressure solvent extraction, Soxhlet, and supercritical extraction using CO_2 and cosolvents. The results showed soxhlet method with ethanol and isopropyl alcohol gave the maximum amount of curcuminoid content, significantly higher than other methods (62).

Pothitirat W. also compared the various methods. Extraction of dried rhizome with hexane to obtain volentile oil before using soxhlet apparatus with 95%ethanol was an appropriate method for extracting curcuminoids from turmeric, when compared the yield of crude, solvent used, extraction time and other costs from other methods. The study applied various methods (solvent extraction, hydrodistillation, precipitation after maceration or using soxhlet apparatus, solvent partition separation) with different solvents (hexane, methanol and petroleum ether) (44).

Different methods provide the difference in yield of curcumin. The affecting factors of the efficiency of solvent extraction are particle size of raw material, extraction medium, extraction temperature and time (41).

3.4.4 Determination of curcuminoid content

Various methods of measurement of curcuminoids extracted from turmeric have been reported such as spectrophotometric absorption method, fluorimetric method, gas chromatography (GC), high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC). Each method may give a different quantitation result depending on their limitation (15, 49).

Most of the previous studies have used the spectrophotometric method to determine the total color content of sample or curcuminoid content that contains curcumin, demethoxycurcumin and bisdemethoxycurcumin. The spectrophotometric method, however, is not appropriate to determine the individual curcuminoid while the HPLC method can produce such results.

3.4.4.1 High-performance liquid chromatography (HPLC)

The analysis of individual curcuminoid is possible by using HPLC on a normal phase or reverse phase C18 column. The common mobile phase solvent, methanol, acetonitrile and tetrahydrofuran (THF) provides a different peak area resolution. The detectors used in several studies were UV-Vis detector and diode array detector. The optimum wavelength used was in the range of 420 – 430 nanometers (20, 23, 44, 63-67)

Jayaprakasha GK *et al.* performed HPLC method using C_{18} column with gradient solvent system at flow rate 1 ml/min, ambient condition. An HP 1100 series variable wavelength detector was used at a wavelength of 425 nm. The mobile phase consisted of methanol, 2% acetic acid and acetonitrile (64, 68).

Ying-Jan Wang M-HP *et al.* used the same method of Jayaprakasha GK but applied a different mobile phase and used a UV-vis detector. Mobile phase of this method comprised of tetrahydrofuran, water and citric acid, pH 3 at ratio (40: 60: 1 %) to determine curcuminoids in methanol solution (24).

Curcuminoids in samples gained from fresh turmeric refluxed with methanol was determined by He X-G *et al.* method. This method used C_{18} colunm with gradient elution using mobile phase containing (A) water (0.25% Acetic acid) and (B) acetonitrile at a flow rate of 0.2 ml/min. A photodiode-array detector was set at 425 nm (for signal A) and 250 nm (for signal B) (20).

Jang H-D *et al.* performed HPLC method using a C_{18} colunm with gradient elution. UV-Vis detector set at 422 nm. The mobile phase for analysis of curcumin was acetonitrile, methanol, deionized water and acetic acid (41:23:36:1, v/v/v/v) at a flow rate of 0.8 ml/min for determining three active principles; curcumin, cinnamaldehyde and berberin concentration (22).

Boonchoong P *et al.* (19) performed HPLC for analyzing active components in turmeric and common Thai herb (Fathalai Jon) capsules by using C_{18} colunm and a UV-Vis detector at 425 nm. Mobile phase was 1% acetic acid and acetonitrile at ratio 44:55 with a flow rate of 1 ml/min. Sample injection was 20 µl.

Morover, HPLC method can be applied with C_{18} reversed phase column as published by Pfeiffer E *et al.* and Price LC and Buescher RW. Pfeiffer E *et al.* (66) used reversed phase column with a linear gradient of acetonitrile in water (from 20% to 70% acetonitrile in 30 min) and used a diode array detector. In the other study, Price LC and Buescher RW (23) method used an isocratic elution at a flow rate of 1.5 ml/min with a mobile phase of THF and water (40:60) and measurement of absorption at 420 nm.

3.5 Antioxidant activities

Halliwell *et al.* (33) defined an antioxidant as "any substance that when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate."

3.5.1 Antioxidant activity determination

Many studies were carried out to test the antioxidant activity of active principles under different cooking conditions and therapeutic applications such as DPPH free radical scavenging assay, ferric-reducing antioxidant power assay (FRAP), ABTS radical cation decolorization assay and other methods.

3.5.1.1 DPPH free radical scavenging assay

DPPH assay uses the stable radical, 1,1-diphenyl-2-picryl-hydrazyl (DPPH) as a reagent. The mechanismcan be described as follows. DPPH free radical acts as an oxidant that reacts with an antioxidant or radical species according to these equations.

$$DPPH^{\cdot} + AH \rightarrow DPPH\text{-}H + A^{\cdot} \quad \text{---- 1}$$

$$DPPH^{\cdot} + R^{\cdot} \rightarrow DPPH - R \quad \text{---- 2}$$

The radical trapping reaction of DPPH with curcumin can be explained with a diagram by Ak T and Glin I. (29). DPPH radical is scavenged by curcumin that donates H atom to form stable DPPH as shown in Figure 11.

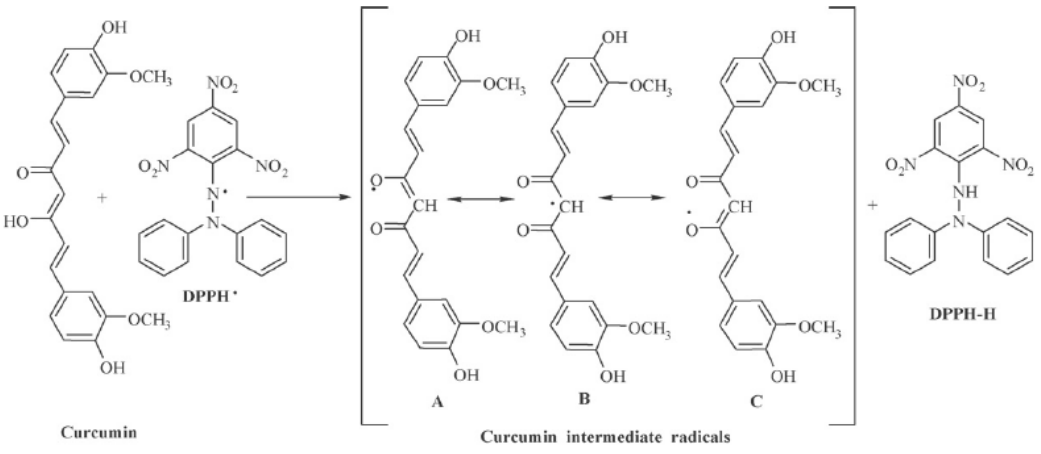

Figure 11 Mechanisms of the radical trapping reactions of DPPH with curcumin (29)

The DPPH radical absorbance is measured by the bleaching of a purple color of DPPH methanol solution at 517 nm (15). When a hydrogen atom and an electron were transferred to an odd electron in DPPH to increase a non-radical form of DPPH, the absorbance is decreased.

The DPPH scavenging capacity in this study was expressed as trolox equivalent antioxidant capacity in mmol/100ml. The procedure is presented in 4.6.2.1 of the Materials and Methods.

3.5.1.2 Ferric-reducing antioxidant power assay (FRAP)

FRAP assay is based on the reduction of ferric (Fe(III)) to ferrous (Fe(II)) form of iron at low pH. In a FRAP mechanism, Fe(III) acts as an oxidant that can be reacted with antioxidant in the sample. Fe(III) is itself reduced to Fe(II) and readily chelates with TPTZ to form Fe(II)-TPTZ complex that gives the color (69).

The absorbance is measured of a colored ferrous-tripyridyltriazine complex (Fe(II)-TPTZ complex) at 593 nm (35). The FRAP antioxidant activity in this study was expressed as trolox equivalent antioxidant capacity in mmol/100ml. The procedure is presented in Section 4.6.2.2 of the Materials and Methods.

3.6 Turmeric colorant

Turmeric colorant is a permit color for food industries. Turmeric has a color that varies from bright-yellow color, very close to the hue of FD&C Yellow No. 5 to reddish brown color upon the application uses (35). Bright-yellow color is due to curcumin, a diketone, (3-7% by weight) in turmeric. This colorant is commonly used as an ingredient for coloring in an emulsified preparation such as mustard, mayonnaise, salad dressings and oils. The turmeric colorant is used in many forms such as curcumin colorant, turmeric powder and turmeric oleoresin. The European Union (EU) permits curcumin colorant (E 100, CI 75300) for use in alcohol beverages, non-alcoholic drinks, jams, jellies, marmalades, dried potato granules and flakes and confectionery. The USFDA approved turmeric powder (CI 73600) and turmeric oleoresin (CI 73615) but not curcumin powder for general use in food. JECFA

established a temporary acceptable daily intake (ADI) at 0 to 1 mg kg/body weight (70).

Curcumin is a natural color that is a choice to replace both quinoline yellow and tartrazine although it is not exactly the same hue (40, 71). Curcumin is not classified as an essential nutrient but has nonetheless been reported to possess potential benefits to human (72). Although curcumin is insoluble in water but it is able to disperse. Curcumin is a bright-yellow food colorant, mainly suited to food applications requiring little or no light stability. The bright-yellow color changes to a faded color with its exposure to the sunlight.

Moreover, curcumin is often used in various products such as bakery product, cereal product, diary product, seafood product, egg product, soft drink and beverage, snack, mustard, confectionery and other products (40).

3.7 Food color appearance

Vision is usually the first sense that people use for detecting the food color and subsequently quality. The process of seeing includes several activities in a stepwise order and the key organs involved are the eyes and the brain. Seeing process is related to many factors such as the surrounding of the object, quality and intensity of light, color appearance and occurred event (34).

3.7.1 Color measurement system

The systems of color measurement in food include many methods as described below.

3.7.1.1 Munsell system

Munsell system is developed in the USA. The chromaticity coordinates in this system are hue, value, and chroma. The comparison standards of Munsell system are provided in the Munsell book of color. Each standard is expressed with an alphanumeric notation. Number takes a value from 1 to 10. A letter is represented to one of ten major hue names such as red (R), yellow (Y), green (G), blue (B), purple (P), red-yellow (RY) (31).

This system shows a high consistency and is rapid, portable, widespread, and economical. Different observers can obtain the same evaluation in similar condition

3.7.1.2 The Commission Internationale de l'Eclairage (CIE L*a*b* system)

The CIE L*a*b* system was modified in 1973. This system has become widely used and now available to use with reflectance spectrophotometric instrumentation. This method measures the reflection and transmission spectrum of an object into a three-dimension color space. This method does not use a real color but seems to base on a trichromatic principle (34). The brightness signal is expressed by lightness (L*) from black to white (0-100). The positive a* value to negative a* value represents redness to greenness. The positive b* value to negative b* value represents yellowness to blueness (36). The color value results from a spectrophotometer are expressed in 3 co-ordinates of L*, a*, b* to define the location in the CIE color space (Figure 12).

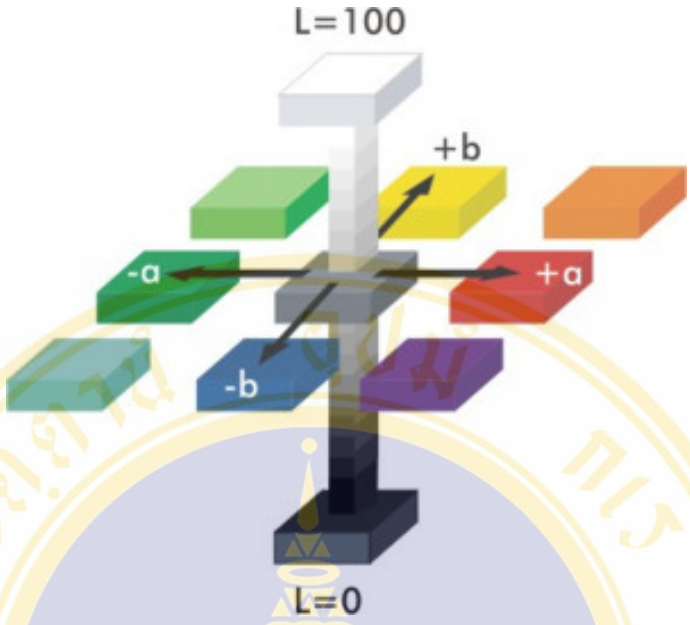

Figure 12 CIE color space (73)

3.8 Toxicity of turmeric

The toxicity study of turmeric has been performed in many countries. Curcumin is poorly absorbed while rapidly excreted. The acute oral toxicity is very low. The oral LD_{50} of curcumin in mice is greater than 2 g/kg whereas that of the oleoresin in rats and mice is greater than 10 g/kg. Feeding 500 mg of turmeric/kg/day to rats for 52 weeks showed no significant toxicity, similar to the results that gained from the experiment with dogs and monkeys. JECFA did not establish an ADI value for turmeric because they considered it as a food. In 1990, a temporary ADI of turmeric oleoresin was established at 0-0.3 mg/kg and 0-0.1 mg/kg for curcumin. Later on the temporary ADI for curcumin was increased to 0-1 mg/kg (31, 34, 70). In medicinal use, high dose of turmeric preparation with anticoagulant drug may increase the risk of bleeding that caution is advised (74).

CHAPTER IV
MATERIALS AND METHODS

4.1 Turmeric by-product

Fresh ground turmeric (*Curcuma longa* L.) pomace, a by-product from a pharmaceutical industry, was supplied by the Thai-China Flavours and Fragrances Industry Co. Ltd. It was used as a raw material for curcuminoid extraction. L*a*b* color value of the turmeric by-product was determined before extraction.

4.2 Preparation of turmeric by-product before extraction

Fresh ground turmeric pomace was washed with tap water at a ratio of 1:2 of fresh ground turmeric: water to remove the remaining starch, water-soluble materials and foreign materials. Because of high water content in the raw material, excess water from the slurry of turmeric pulp was separated using a nylon bag with a hydraulic press. The pulp was repeatedly washed and removed excess water. The turmeric pulp was kept in polyethylene bags in a freezer at -20°C until extraction of curcuminoids.

4.3 Carotenoid determination in turmeric by-product

Turmeric pulp was homogenized to prepare a homogeneous sample. The homogenized sample was sent to the analytical laboratory of the Institute of Nutrition, Mahidol University to determine the carotenoid content in turmeric by-product. This analysis was carried out to determine whether carotenoids were present in a significant amount and should be followed in the storage study.

4.4 Preparation of fresh turmeric pulp for analysis

Turmeric pulp prepared from the by-product, was homogenized in a blender. Five hundred mg of turmeric pulp were weighed into 50 ml centrifuge tubes with cap and added with 30 ml 95% ethanol into each tube. The centrifuge tube was shaken with a rotary shaker at 100 rpm at room temperature for 1 h. After that the samples were centrifuged at 3000 rpm for 5 min. Supernatant was collected and kept at 5°C until analysis.

4.5 Extraction of curcuminoids from turmeric pulp to produce turmeric colorant

Turmeric pulp was extracted with 95% ethanol at a ratio of 1:5 (kg wet weight turmeric pulp: liter of alcohol) to extract the curcuminoids. The extraction was carried out in a plastic bucket with continuous agitation by a mechanical propeller for 16-18 h. The ethanolic extract (liquid part) was filtered using a gauze fiber then passed through a Whatman No.42 filter paper to remove macro materials such as starch. The extract was evaporated using a rotary evaporator at 40°C to make five fold curcuminoid-rich concentrate. The curcuminoid-rich concentrate (turmeric colorant) was kept in amber glass bottles with screw cap and sealed with parafilm.

4.6 Storage study of turmeric colorant

Turmeric colorant was kept under different storage conditions to determine the optimum condition for storage. The study was performed in 2 parts; to study the effect of temperature and addition of a protective agent and to follow curcuminoid content, antioxidant activity and the color of the turmeric colorant during 2 months.

4.6.1 Effect of temperature during storage

Ten ml each portions of turmeric colorant were placed in 42 brown bottles and flushed with nitrogen gas before placing the caps. One set of twenty one bottles was stored in the dark at 4°C and another set at room temperature (about 25°C - 27°C)

for 2 months. Three bottles from each set was randomly removed 7 times at 0 day, 3 days, 1 week, 2 weeks, 4 weeks, 6 weeks and 8 weeks for determining curcuminoid concentration and color measurement. Antioxidant activity of the colorant was determined 6 times at 0 day, 1 week, 2 weeks, 4 weeks, 6 weeks and 8 weeks.

4.6.2 Effect of adding a protective agent (dl-α-Tocopherol)

For the storage study at room temperature (about 25°C - 27°C), another set of colorant was added with a protective agent to determine whether it would help preserve the quality of the colorant. Prior to filling into brown bottles as stated in Section 4.6.1 above, one set of twenty one bottles of turmeric colorant was added with dl-α-Tocopherol at a concentration of 400 ppm. This third set was stored at room temperature for 2 months. During storage the sample was drawn for determining curcuminoid concentration, color measurement and antioxidant activity using the same protocol as above

4.7 Analytical methods

Fresh turmeric pulp and all randomized samples of extracted turmeric colorant at different storage time were analyzed for their curcuminoid concentration, antioxidant activity and L*a*b* color parameter measurement.

4.7.1 Curcuminoid concentration determination by HPLC analysis

4.7.1.1 Standard preparation

The HPLC grade standard curcuminoid used in this study was standard curcumin (Fluka, 28260) that contains a mixture of curcumin, demethoxycurcumin and bisdemethoxycurcumin. The standard was freshly prepared by dissolving in HPLC mobile phase that consists of 1% acetic acid and acetonitrile (45:55 v/v) to prepare curcuminoid stock solution 1000 µg/ml. Curcuminoid stock solution was pipetted and diluted with mobile phase to prepare a serial dilution in the range of concentration 100, 50, 30, 20, 10, 5 µg/ml. The calibration curve of standard curcumin was linear with R^2 value exceeded 0.95. The HPLC procedure is shown in Appendix A (19).

4.7.1.2 Fresh turmeric sample preparation for HPLC analysis

The collected supernatant from fresh turmeric pulp in Section 4.4 was diluted with 95%ethanol to make an appropriate concentration for HPLC analysis then passed through a Whatman syringe filter (Nylon, 0.2 μm). Samples were kept in micro centrifuge tubes in the dark before injection.

4.7.1.3 Turmeric colorant preparation for HPLC analysis

Randomized turmeric colorant sample was analyzed for curcuminoid content using HPLC. Turmeric colorant was diluted with 95%ethanol to an appropriate concentration for HPLC analysis and then passed through a Whatman syringe filter (Nylon, 0.2 μm) before injection. HPLC data were collected to compare curcuminoid content among fresh turmeric pulp and turmeric colorant.

4.7.1.4 HPLC analysis

Curcuminoid analysis of all samples using HPLC was performed in triplicates. The curcuminoid concentration was determined on an HPLC system equipped with a Waters 510 HPLC pump and a Hewlett Packard series 1050 UV-Visible detector at wavelength 425 nm. The method used was a modified method of Boonchoong P and Saohin W (14). A sample of 20 μl was injected into a C_{18} reverse phase column (250 x 4.6 mm, 5μm particle size, Econosphere) and eluted with a mobile phase containing 1% acetic acid and acetonitrile (45:55 v/v) at a flow rate of 1 ml/min. Total peak area of three active compounds (curcumin, demethoxycurcumin and bisdemethoxycurcumin) was calculated using Chemstation Software (Aligate Technologies, USA). Standard curcumin (Fluka, 28260) was used as a reference. The result was expressed as total curcuminoids (mg/ml). The HPLC chemical preparation and procedure are shown in Appendix A.

4.7.2 Determination of antioxidant properties

The antioxidant properties were determined in triplicates using DPPH free radical scavenging assay and ferric-reducing antioxidant power assay (FRAP) The measurement for colorant was performed 6 times at storage time 0 day, 1 week, 2 weeks, 4 weeks, 6 weeks and 8 weeks.

4.7.2.1 DPPH free radical scavenging assay determination

The DPPH free radical scavenging assay was performed using a modified method of Burda S and Oleszek W (75). Two milliliters of DPPH ethanol solution was reacted with 1 ml of diluted sample or standard solution in the dark at room temperature for 30 min. Trolox ethanol solution was used for standard curve. The absorbance was measured at 517 nm using a spectrophotometer. The absorbance data were calculated for the DPPH activity and the results were expressed in term of Trolox equivalent antioxidant capacity (TE) unit (mmol/100ml). DPPH assay procedure is given as Appendix B.

4.7.2.2 Ferric-reducing antioxidant power assay (FRAP)

FRAP assay was performed using a modified method of Benzie IFF and Strain JJ (76). One ml of each diluted sample solution was mixed with 3 ml FRAP reagent. The sample was incubated at 37°C for exactly 4 min in the dark. Deionized water was used as control. Trolox solution was used for calibration. The absorbance was measured at 593 nm using a spectrophotometer. The FRAP antioxidant power activity was expressed in term of Trolox equivalent antioxidant capacity (TE) unit (mmol/100ml). FRAP assay procedure is given as Appendix C.

4.7.3 Color measurement

Color value was measured in triplicates using a spectro-colorimeter model JS555 (Color Techno System Corporation, Tokyo, Japan). Two hundred and fifty microliters of turmeric colorant was diluted in 50 ml deionized water or 95% alcohol before measurement. The value was detected by reflective detection and expressed as L*, a*, b*. The L* value represented lightness, a* and b* values represent redness and yellowness, respectively. Tungsten halogen lamp was used as a light source.

4.8 Study of the L*a*b* color parameter of turmeric colorant at different pH

Two hundred and twenty five microliters of turmeric colorant were dissolved in 50 ml solutions at varying pH values which contained either hydrochloric acid or sodium hydroxide. The colorant was dissolved in the solutions in the range of pH 3, 5, 7, 9, 11 to study color shading. L*a*b* color parameters at different pH conditions were determined in five replicates using the same method as described in Section 4.7.3.

4.9 Application of turmeric colorant in food products

Eight food samples that commonly appear yellow in color from different food categories e.g. prepared with dry heat or moist heat, contained high moisture or low moisture in the final product, prepared with oil, were used as models to study the application of turmeric colorant in food products. Eight examples of these foods include three Thai desserts (Kanom num dok mai, Bua loy num kati, and Glossy sticky rice), butter cream for cake decoration, fried rice crispy, orange flavored ice confection, orange flavored jelly and orange flavored soft drink. Recipes of the eight food samples are shown in Appendix F.

4.9.1 Preparation of food products

a. Glossy sticky rice -Thai dessert

Glossy sticky rice formula was adapted from a Thai dessert recipe book. Glutinous rice was soaked in water for 4 h before cooked by steaming for 30 min to make sticky rice. Sticky rice was added with sugar and coconut milk and separated into 2 equal batches. One batch was added with tartrazine while another added with turmeric colorant. Each batch was boiled and stirred for 30 min until became glossy and almost dry (low moisture). Glossy sticky rice was filled into a mold to harden and stored in a plastic container at room temperature.

b. Kanom num dok mai (steamed rice cake) - Thai dessert

Sugar was added to water then boiled to make syrup. A starch mix comprising of rice starch and rough starch was kneaded with little added water until all water was absorbed. Cooled syrup was added to the starch mix in a bowl. The liquid starch mixture was separated into 2 batches. One batch was added with tartrazine while another added with turmeric colorant. Each batch of liquid starch mixture was filled and steamed in small porcelain cups on boiling water for 10 min. Cooked rice cake was picked out from cup and stored in a plastic container at room temperature.

c. Bua loy num kati (Rice balls in coconut milk) - Thai dessert

Bua loy num kati formula was adapted from a Thai dessert recipe book. Glutinous rice flour was kneaded with water to make a dough. The dough was separated into 2 batches. One batch was added with tartrazine while another added with turmeric colorant. The colored dough was then manually shaped into small globular rice balls and cooked in boiling water. Coconut milk, sugar and salt were mixed and boiled. Cooked coconut milk and rice balls were separately stored in polypropylene bags at room temperature. Cooked rice balls were added into the cooked coconut milk for 5 min before served.

d. Butter cream for cake decoration

Syrup, boiled mixture of sugar and water, was cooled down in a refrigerator. Butter and shortening were added into a mixing bowl of an electric mixer and blended at a low speed until soft and homogenous. Part of sugar was gradually blended in using a medium speed after each addition until homogenous. Butter cream was separated into two batches. One batch was added with tartrazine while another added with turmeric colorant. Butter cream cake was filled in plastic cups and stored at 4°C in a refrigerator.

e. Fried rice crispy

Tapioca starch was added with salt, garlic and pepper and kneaded with hot water manually. The resulting dough was separated into 2 batches. One batch was added with tartrazine while another added with turmeric colorant. The colored dough was formed into a bar shape and steamed over boiling water. The

cooked dough was chilled at 4°C in a refrigerator for 12 hours then sliced into pieces and sun dried. Dried rice pieces were deep-fried in palm oil, drained, cooled and stored in polypropylene bags at room temperature.

f. Orange flavored soft drink

Drinking water, fructose syrup, sugar and citric acid were mixed together in two batches. Both batches of soft drink were added with sunset yellow to give a red-orange color shade. Then the yellow color shade in one batch was given by tartrazine while another batch was replaced with turmeric colorant. Titanium dioxide was added to each batch to give turbidity. The soft drink was kept in a polyethylene terephthalate bottle at 4°C in a refrigerator.

g. Orange flavored jelly

Drinking water, fructose syrup and sugar were mixed. The solution was boiled and added with agar until clear and stirred with citric acid. Both batches of agar solution were added with sunset yellow to give a red-orange color shade. Then the yellow color shade in one batch was given by tartrazine while another set was replaced with turmeric colorant. Orange flavor jelly was filled in plastic cups and stored at 4°C in a refrigerator.

h. Orange flavored ice confection

Drinking water, fructose syrup, sugar and citric acid were mixed together and divided into two batches. Both batches of mixture were added with sunset yellow to give a red-orange color shade. Then the yellow color shade in one batch was given by tartrazine while another batch was replaced with turmeric colorant. Titanium dioxide was added to each batch to give turbidity. The mixture was filled in plastic cups, frozen and kept at -20°C in a freezer.

4.9.2 Determination of color of food products

Two samples of each kind of foods, one added with turmeric colorant and another added with commercial synthetic colorant such as tartrazine and sunset yellow, were determined for their L*a*b* color parameters by using a spectro-colorimeter model JS555 to compare the difference in color between the two samples.

Butter cream for cake decoration, fried rice crispy, orange flavored jelly, Kanom num dok mai, Bua loy num kati, glossy sticky rice and orange flavored ice

confection were measured L*a*b* color parameters in triplicates by reflectance method.

Orange flavored soft drink was measured L*a*b* color parameters in triplicates by transmittance method.

4.9.3 Sensory evaluation test of turmeric color added food products

Sensory evaluation was performed to test the effect of turmeric colorant on the consumer's acceptance of the foods compared with samples added with synthetic colorants in terms of color and odor acceptability. Each sample was evaluated by 30 untrained panelists who are staff and graduate students at the Institute of Nutrition, Mahidol University. For sensory evaluation, a randomized complete block design (RCB) was used (block by panelists) to evaluate the color of food added with turmeric colorant compared with samples added with synthetic colorants.

Food samples were prepared one day before evaluation, packed in suitable packages and stored at a common storage temperature for each product as described in Section 4.9.1.

Each food sample, coded with a three-digit random number obtained from a random number table, was placed in a clear plastic cup on a white plate. The sample was randomized in serving to the panelists. The evaluation was performed in air-conditioned testing booths under a daylight fluorescent lamp at a sensory laboratory of the Institute of Nutrition, Mahidol University. Sensory evaluation was performed using 9-point hedonic scales for acceptability test while difference test was performed using 15-cm line scales (77) as shown in Appendix G.

4.10. Statistical analysis

All analyses were performed in triplicates (n=3). The data were analyzed with an SPSS commercial statistical software package (version 13.0) to distinguish significant differences among groups at 5% level of probability. The results were expressed as mean ± standard deviation (S.D.).

For curcuminoid concentration, antioxidant capacity (DPPH and FRAP), L*a*b* color parameter results one way ANOVA was used, followed by the Duncan

test with significant difference ($p \leq 0.05$) to compare the difference among different storage time and condition.

For sensory evaluation, a randomized complete block design (RCB) was used (block by panelists) to evaluate the effect of added color to food products comparing turmeric colorant with synthetic colorants. Eight food samples added with turmeric colorant and eight food samples added with synthetic colorants were evaluated by 30 untrained panelists. A paired T-test was used to establish the significant difference by choosing p-value 0.05. The data were analyzed with an SPSS commercial statistical software package (version 13.0).

CHAPTER V
RESULTS

5.1 Turmeric pomace

A photograph of turmeric pulp from fresh turmeric promace after separating excess water appears as Figure 13. The turmeric pulp was kept in polyethylene bags in a freezer at -20°C until extraction of curcuminoids. L*a*b* color parameters were determined before extraction. The results are shown in Table 4.

Figure 13 Turmeric pulp after washing and separating excess water

Table 4 L*a*b* color parameters of turmeric pulp [1]

Color parameter	Value
L*	38.43±0.18
a*	11.28±0.30
b*	44.92±1.04

[1] Results expressed as mean ± SD of five replicate analyses

5.2 Carotenoid determination in turmeric by-product

The analytical result from the analytical laboratory of the Institute of Nutrition, Mahidol University showed that the β-carotenoid content in turmeric pulp was 17.78 µg/100g (according to the report no. SFC 1722/2551 in Appendix D). Turmeric pulp contained only a small amount of carotenoids, therefore a change in carotenoid content was not followed after extraction and storage of the turmeric colorant.

5.3 Extraction of curcuminoids from turmeric pulp to produce turmeric colorant

5.3.1 Effect of evaporation for turmeric colorant extraction

Eight hundred g of turmeric pulp were extracted with 4000 ml 95% ethanol to obtain a turmeric extract. In the first trial, the liquid extract was evaporated using a rotary evaporator to make a ten-fold concentrated colorant. The resulting product was found to be unsuitable for use in general because it was mixed with high amount of wax as oleoresin and was difficult to disperse in many common solvents such as ethanol and deionized water. In subsequent trials the turmeric extract was then prepared at five-fold concentration instead of ten-fold to obtain a liquid colorant that was more easily dispersible.

5.3.2 Yield of extraction

The curcuminoid content of turmeric colorant (concentrated extract from Section 5.3.1) measured by HPLC analysis was 7.95±0.95 g in the total extract from 800 g fresh turmeric by-product. Curcuminoid concentration of turmeric colorant was lower than that in fresh turmeric by-product (18.60 ±0.95 mg/g or 14.88 g in 800 g). Thus, the yield of curcuminoid extraction was 53.4%.

5.3.3 Appearance of turmeric colorant

Turmeric colorant exhibited a dark brown color because of it was concentrated. Turmeric colorant contained a mixed form of curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin) and ethanol. The photograph of turmeric colorant is shown in Figure 14.

(a) (b)

Figure 14 Turmeric colorant extracted from turmeric by-product of pharmaceutical industry; (a) colorant in bulk solution and (b) one drop of colorant.

5.4 Storage study of turmeric colorant

Turmeric colorant was kept under different storage conditions. HPLC analysis, DPPH free radical scavenging assay and ferric-reducing antioxidant power assay (FRAP) were performed to study the effect of temperature and addition of a protective agent by following curcuminoid content, antioxidant activity and the color of the turmeric colorant during 2 m of storage.

5.4.1. Curcuminoid concentration in turmeric colorant kept under different storage conditions

The results of triplicate HPLC analyses of samples are illustrated in Figure 15 and the data are included in Appendix H. An example of a standard curcuminoid chromatogram appears in Appendix E. The average curcuminoid concentration in turmeric colorant on the first day (0 day of storage) ranged from 9.03±1.33 to 10.75±0.63 mg/ml (average= 9.9±1.2 mg/ml).

For storage at 4°C, curcuminoid concentration was 10.02±1.04 mg/ml on the first day and tended to decrease slightly to 7.81±0.86 mg/ml within the first two weeks. Curcuminoid concentration changed to 8.48±0.50 mg/ml in the fourth week and to 8.20±1.57 mg/ml in the last determination of the 2-month storage. However, there was no significant difference among all values ($p>0.05$).

For storage at room temperature, curcuminoid concentration was 9.03±1.33 mg/ml on the first day of storage. Curcuminoid concentration tended to decrease to 7.59±1.21 mg/ml within the first two weeks and slightly increased to 9.00±0.90 mg/ml in last determination of the 2-month storage. No significant difference was found among all values ($p>0.05$).

For storage at room temperature with dl-α-Tocopherol, curcuminoid concentration was 10.75±0.63 mg/ml on the first day of storage and decreased to 7.88±0.31 mg/ml in the first week. Then curcuminoid concentration slightly changed to 9.50±0.74 mg/ml in the fourth week and to 8.84±0.65 mg/ml in the last determination of the 2-month storage. There was no significant difference among all values ($p>0.05$).

5.4.1.1 Effect of temperature

Temperature exhibited a slight effect on the amount of curcuminoids in turmeric colorant. Storage at 4°C and room temperature seemed to give similar results with no significant difference ($p>0.05$) among all values during the 2-month storage.

5.4.1.2 Effect of addition of a protective agent

The protective agent used in this study, dl-α-Tocopherol showed a slight effect on the amount of curcuminoids in turmeric colorant. Storage with added dl-α-Tocopherol seemed to give better results than without added dl-α-Tocopherol at the same storage temperature. However, no significant difference ($p>0.05$) was found.

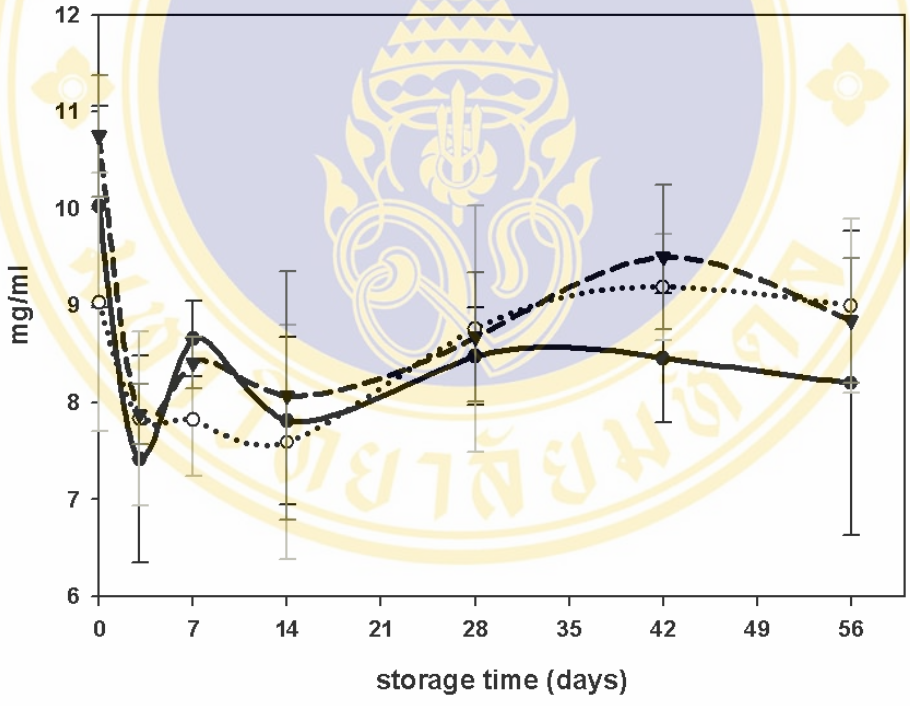

Figure 15 Curcuminoid concentration in turmeric colorant during storage at different conditions: —●— storage at 4°C, ···○··· storage at room temperature and —▼— storage at room temperature with dl-α-Tocopherol

5.4.2 Antioxidant properties of turmeric colorant

DPPH and FRAP assays were selected to determine antioxidant activity of the extracted turmeric colorant in this study because they are inexpensive, simple to prepare and widely used (22, 25, 29).

5.4.2.1 DPPH free radical scavenging assay

The antioxidant capacity of turmeric colorant was determined by DPPH assay in six replicates. The results are illustrated in Figure 16 and the data are shown in Appendix I. The average DPPH antioxidant capacity on the first day (0 day of storage) was 3.04±0.19 mmol/100ml.

Storage under all conditions gave fluctuated results during the 2-month time period, the DPPH antioxidant capacity of turmeric colorant varied up and down within one unit of the TEAC value. Although significant differences ($p \leq 0.05$) were found between certain points along the storage, the overall changes in the TEAC value were small. Moreover, for storage at room temperature with dl-α-Tocopherol, no significant difference ($p > 0.05$) was found during the entire storage period.

5.4.2.1.1 Effect of temperature

Temperature exhibited a slight effect on the DPPH antioxidant capacity of curcuminoids in turmeric colorant. Storage at 4°C and room temperature seemed to give similar results although significant differences ($p \leq 0.05$) were found at certain points during the 2-month storage. Overall, storage at either 4°C or room temperature should be possible.

5.4.2.1.2 Effect of a protective agent

Addition of a protective agent had no effects on the DPPH antioxidant capacity in turmeric colorant. Storing the colorant with and without dl-α-Tocopherol showed no significant difference ($p > 0.05$) throughout the 2-month storage period.

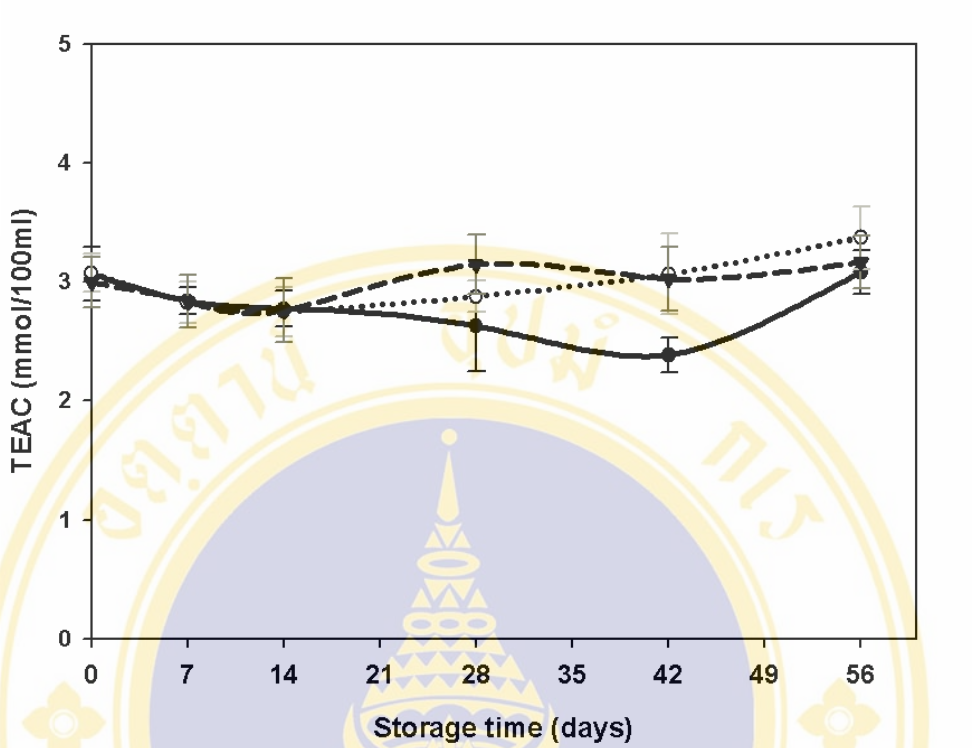

Figure 16 Antioxidant capacity of turmeric colorant determined by DPPH assay during storage at different conditions: —●— storage at 4°C, ···○··· storage at room temperature and —▼— storage at room temperature with dl-α-Tocopherol

5.4.2.2 Ferric-reducing antioxidant power assay (FRAP)

The antioxidant capacity of turmeric colorant was confirmed by FRAP assay in six replicates. The results are shown in Figure 17 and the data appear in Appendix J. The average FRAP antioxidant capacity on the first day (0 day of storage) was 2.92±0.17 mmol/100ml.

Similar to the DPPH antioxidant study, storage under all conditions gave fluctuated results during the 2-month time period, the FRAP antioxidant capacity of turmeric colorant also varied up and down within one unit of the TEAC value. For storage at 4ºC although significant differences ($p \leq 0.05$) were found between certain points along the storage, the overall changes in the TEAC value were small. Nevertheless, for storage at room temperature the FRAP antioxidant capacity of turmeric colorant seemed to decrease significantly ($p \leq 0.05$) after one week storage and did not significantly change further ($p > 0.05$) until the end of the storage period.

5.4.2.2.1 Effect of temperature

Temperature had a slight effect on the FRAP antioxidant capacity in turmeric colorant. The results of storage at 4ºC showed a similar trend to storage at room temperature with significant difference ($p \leq 0.05$) found on some sampling days. Overall, storage at either 4°C or room temperature should be possible.

5.4.2.2.2 Effect of a protective agent

Addition of a protective agent showed a slight effect on the FRAP antioxidant capacity in turmeric colorant. Storage with and without dl-α-Tocopherol gave similar results although significant differences ($p \leq 0.05$) were found during the first two weeks of storage.

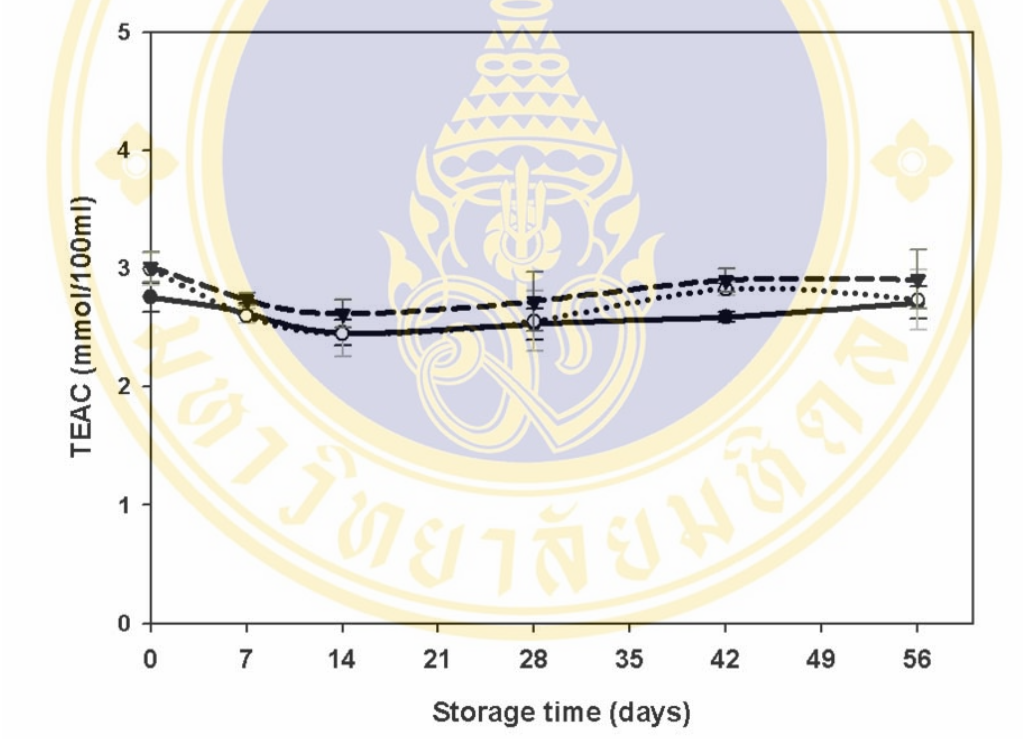

Figure 17 Antioxidant capacity of turmeric colorant determined by FRAP assay during storage at different conditions: —●— storage at 4°C, ···○··· storage at room temperature and —▼— storage at room temperature with dl-α-Tocopherol

5.4.3 CIE L*a*b* color parameters measurement of turmeric colorant

Turmeric colorant was dissolved in two different solvents; 95% ethanol and deionized water before measured L*a*b* color parameters by using a spectro-colorimeter.

5.4.3.1 CIE L*a*b* color parameters of turmeric colorant dissolved in 95%ethanol (Figure 18 and Appendix K).

For L* value (brightness): All turmeric colorant samples kept at three different conditions showed a similar trend of change. From the results the overall L*value of the turmeric colorant stored under all three conditions increased slightly but significantly ($p \leq 0.05$) from 93 to 95.

For a* value (greenness-redness): Turmeric colorant samples from all three storage conditions showed an increasing trend of a* value at the beginning. After that the value decreased slightly but significantly ($p \leq 0.05$) until the end of the experiment. The a* values were in the range of -21 to -17 which correspond to green color.

For b* value (blueness-yellowness): All turmeric colorant samples, stored under different conditions, exhibited a decreasing trend of b* value in the range of 114 to 101 which correspond to yellow color. Significant differences ($p \leq 0.05$) were found among different storage time and temperature.

However, it should be noted that the shade of turmeric colorant dissolved in 95%ethanol from all storage conditions did not appear to be clearly distinguishable by visual observation as presented in Figure 19.

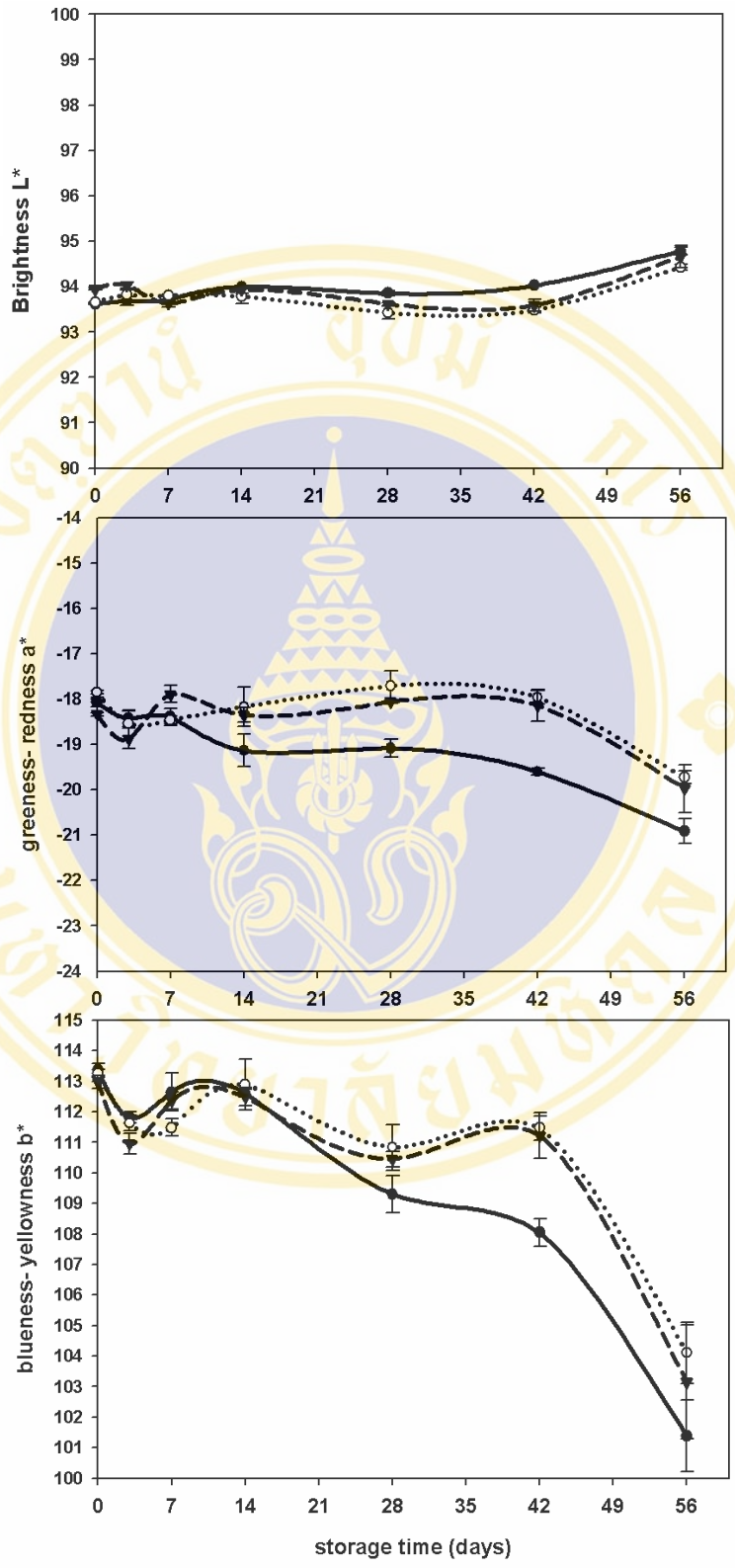

Figure 18 L*a*b*color parameters of turmeric colorant dissolved in 95% ethanol from different storage conditions (storage at 4°C, storage at room temperature, and storage at room temperature with dl-α-Tocopherol).

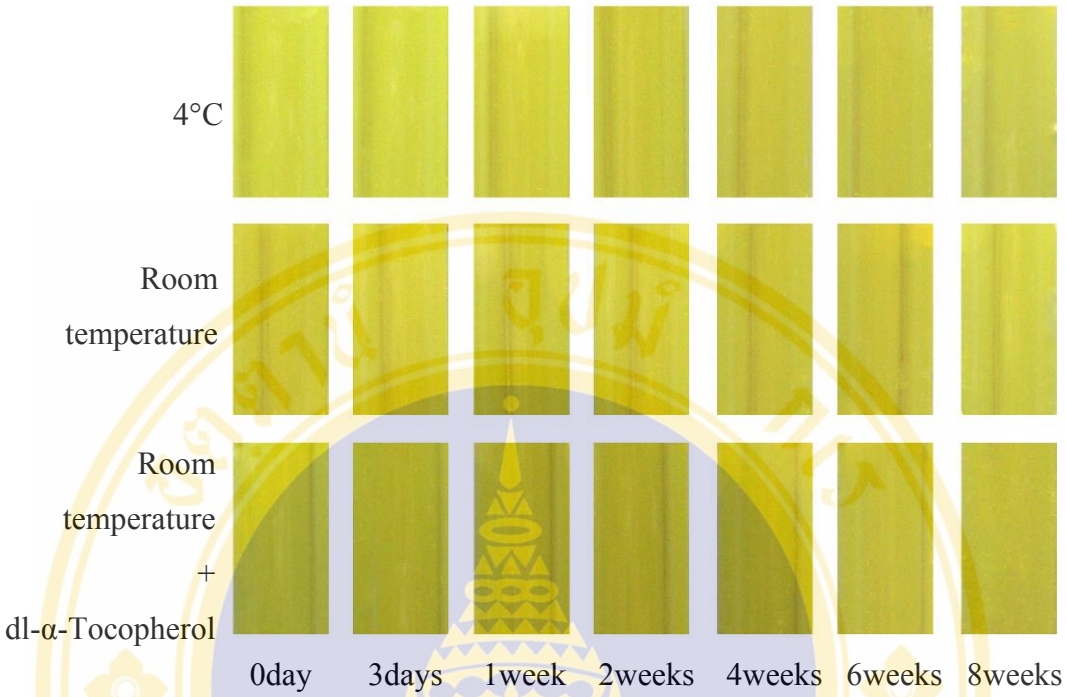

Figure 19 Two hundred and fifty µl turmeric colorant dissolved in 50 ml of 95%ethanol from different storage condition and time

5.4.3.2 CIE L*a*b* color parameters of turmeric colorant dissolved in deionized water (Figure 20 and Appendix L)

For L* value (brightness): All turmeric colorant samples stored under different conditions showed an increasing trend. The values were in a range of 58 to 75 with significant difference ($p \leq 0.05$) among samples from different storage temperature at certain points of the 2-month period.

For a* value (greenness-redness): All turmeric colorant samples, stored under different conditions, significantly decreased ($p \leq 0.05$) in a* value with increasing time. The a* values were in the range of 8 to 22 (redness). Significant difference in the a* value among samples stored at different temperature was found at certain points of the 2-month period.

For b* value (blueness-yellowness): All turmeric colorant samples showed an increasing trend of b* value during the first three days of storage. After that only small changes were observed. The values ranged from 99 to 115 (yellowness).

Similar to the above results of turmeric colorant in ethanol, the shade of turmeric colorant dissolved in 95%ethanol from all storage conditions did not appear to be clearly distinguishable by visual observation as presented in Figure 21.

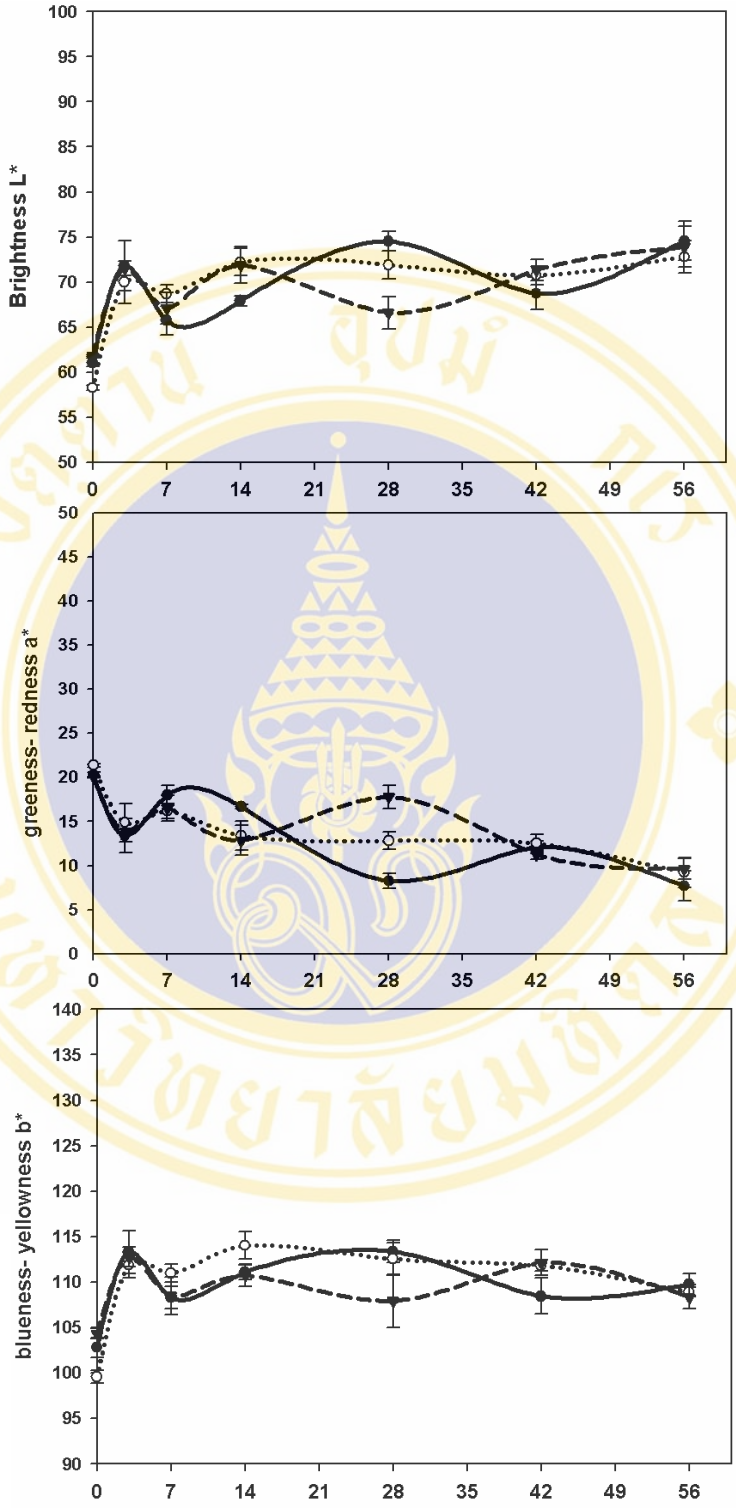

Figure 20 L*a*b*color parameters of turmeric colorant dissolved in deionized water (250µl in 50 ml) from different storage conditions. (—●— storage at 4°C, ···○··· storage at room temperature, and —▼— storage at room temperature with dl-α-Tocopherol)

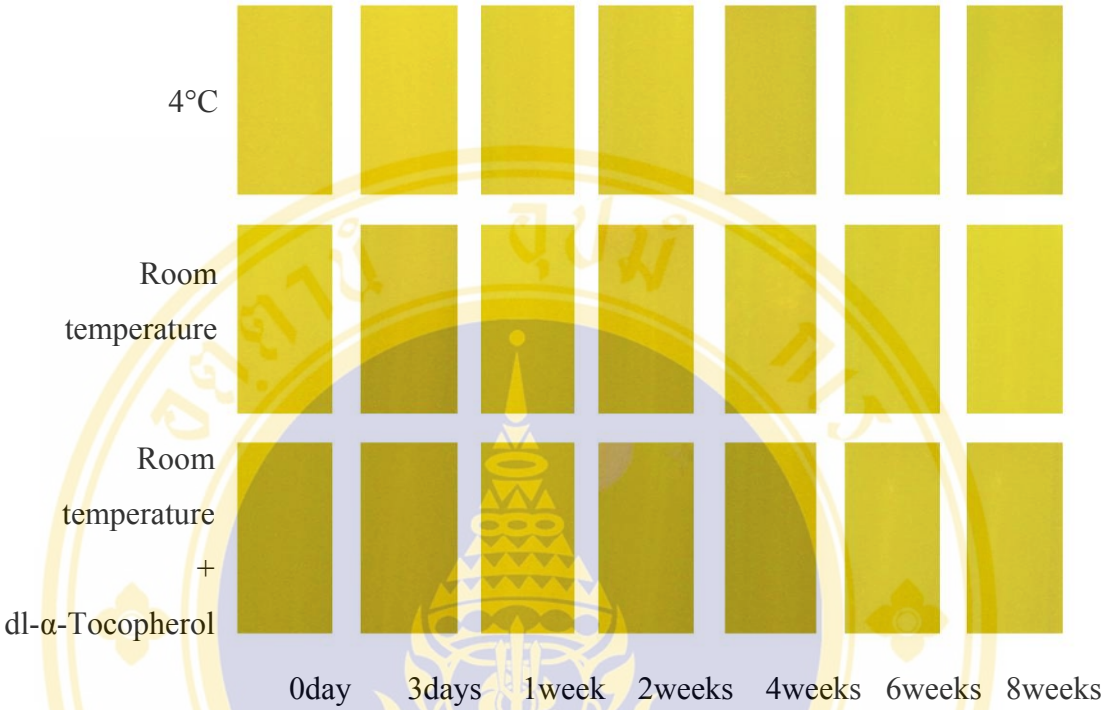

Figure 21 Two hundred and fifty µl turmeric colorant dissolved in 50 ml deionized water from different storage condition and time

5.5 CIE L*a*b color parameters of turmeric colorant at different pH values

L*a*b* color parameters of dissolved turmeric colorant in deionized water were measured to determine the color at different pH values that may be encountered in common foodstuff. The results are shown in Appendix M. All three parameters (L*, a* and b*) changed significantly ($p \leq 0.05$) among varying pH values. The L* value decreased when the solution became more basic while the a* and b* values increased. Nevertheless, by visual observation the color of the solutions at pH 4 to 9 was not noticeably different. All appeared yellow in color. On the other hand, the solution at pH 10 appeared orange-yellowish as presented in Figure 22.

Figure 22 The color of turmeric colorant solutions from pH 4 to pH 10.

5.6 Application of turmeric colorant in food products

5.6.1 Color measurement, appearance and consumer acceptance.

a. Glossy sticky rice - Thai dessert

All CIE L*a*b* color parameters (Table 5) of tartrazine added glossy sticky rice significantly differed ($p \leq 0.05$) from those of turmeric colorant added sample. The L*, a* and b* values of synthetic colorant added sample were higher than turmeric colorant added sample. The appearance of both samples of glossy sticky rice was yellowish in color as shown in Figure 23.

In sensory evaluation (Table 6), the panelists liked the color of turmeric colorant added sample moderately to very much with the score of 7.50±1.57 which was similar ($p > 0.05$) to that of synthetic colorant sample (7.37±1.27). The results were the same for odor acceptance with the score for turmeric colorant added sample and synthetic colorant sample being 7.43±1.07 and 7.50±1.36, respectively. The mean value of color and odor difference score among paired samples was 6.47±3.55 and 4.87±3.67, respectively. Both sets of score had a mode value equaled to 4 which indicated that use of turmeric colorant in glossy sticky rice differed only slightly from use of synthetic colorant.

Figure 23 Glossy sticky rice with tartrazine (left) and turmeric colorant (right)

Table 5 Comparison of L*a*b* color parameters of synthetic colorant added and turmeric colorant added glossy sticky rice.

Colorant	CIE L*a*b* color parameters		
	L*	a*	b*
Tartrazine	56.97±0.56 [b]	-6.68±0.19 [b]	37.31±0.59 [b]
Turmeric colorant	56.09±0.48 [a]	-10.00±0.18 [a]	35.46±1.23 [a]

[1] Results are mean ± SD of three batches with triplicate analysis (n=9).
[2] Value in vertical with different superscript (a,b), Paired-sample t t-test was used with significant different ($p \leq 0.05$).

Table 6 Comparison of acceptance and difference scores (color and odor) of glossy sticky rice

Evaluation	Sample no.	
	821 (Tartrazine)	582 (Turmeric colorant)
Color acceptance score	7.37±1.27[a]	7.50±1.57[a]
Odor acceptance score	7.50±1.36[a]	7.43±1.07[a]
Color difference score (n=30)		
Mean	6.47±3.55	
Mode	4 (Slight difference)	
Odor difference score (n=30)		
Mean	4.87±3.67	
Mode	4 (Slight difference)	

[1] Results are sensory score (mean ± SD) with 30 untrained panelists.
[2] Value of color and odor acceptance in horizontal with different superscript (a,b), Paired-sample t-test was used with significant different ($p \leq 0.05$).
[3] Difference evaluation of color and odor among pair of sample was evaluated by 15 centimeters long scale to calculate the average of score (mean ± SD) with scale 0=no difference, 0.01-3.75 = slight difference, 3.76-7.50 = moderate difference, 7.51-11.25 = large difference, 11.26-15 = very large difference.

b. Kanom num dok mai (rice cake) - Thai dessert

Tartrazine added Kanom num dok mai did not significantly differ ($p>0.05$) from turmeric colorant added sample in the L* value but was higher in the a* and b* values with significant difference ($p\leq0.05$) (Table 7). Both samples showed yellowish color with tartrazine giving a brighter color. The appearance of both samples of Kanom num dok mai is shown in Figure 24.

In sensory evaluation (Table 8), the panelists moderately to very much liked the color of the tartrazine added sample with the score being 7.47±0.82. It was not significantly different ($p>0.05$) from turmeric colorant added sample (7.27±1.11). Similarly, the odor acceptance score of tartrazine and turmeric added sample was moderately liked (6.80±1.42 and 6.73±1.60, respectively). The average color and odor difference score among paired samples was 3.40±2.33 and 3.43±3.11, respectively. The mode value of both sets of scores was 4 which indicated that most panelists detected turmeric added rice cake as slightly different from tartrazine added rice cake.

Figure 24 Kanom num dok mai with tartrazine (left) and turmeric colorant (right)

Table 7 Comparison of L*a*b* color parameters of synthetic colorant added and turmeric colorant added Kanom num dok mai.

Colorant	CIE L*a*b* color parameter		
	L*	a*	b*
Tartrazine	69.18±0.73[a]	-10.26±0.14[b]	45.19±0.46[b]
Turmeric colorant	69.14±0.41[a]	-12.01±0.13[a]	42.64±0.66[a]

[1] Results are mean ± SD of three batches with triplicate analysis (n=9).
[2] Value in vertical with different superscript (a,b), Paired-sample t t-test was used with significant different ($p \leq 0.05$).

Table 8 Comparison of sensory and difference scores (color and odor) of Kanom num dok mai

Evaluation	Sample no.	
	387 (Tartrazine)	355 (Turmeric colorant)
Color acceptance score	7.47±0.82[a]	7.27±1.11[a]
Odor acceptance score	6.73±1.60[a]	6.80±1.42[a]
Color difference score (n=30)		
Mean	3.40±2.33	
Mode	4 (Slight difference)	
Odor difference score (n=30)		
Mean	3.50±3.18	
Mode	4 (Slight difference)	

[1] Results are sensory score (mean ± SD) with 30 untrained panelists.
[2] Value in horizontal with different superscript (a,b), Paired-sample t-test was used with significant different ($p \leq 0.05$).
[3] Difference evaluations among pair of sample was evaluated by 15 centimeters long scale to calculate the average of score (mean ± SD) with scale 0=no difference, 0.01-3.75 = slight difference, 3.76-7.50 = moderate difference, 7.51-11.25 = large difference, 11.26-15 = very large difference.

c. Bua loy num kati (Thai rice balls in coconut milk) - Thai dessert

The L* and a* values of tartrazine added Bua loy num kati were significantly higher ($p \leq 0.05$) than those of turmeric colorant added sample while there was no significant difference ($p > 0.05$) in the b* value (Table 9). Both colorant added samples showed yellowish color .The appearance of both samples of Bua loy num kati is presented in Figure 25.

In sensory evaluation (Table 10), the panelists liked the color and odor of the turmeric colorant added sample moderately to very much with the score of 7.50±1.20 and 7.10±1.16, respectively. There was no significant difference ($p > 0.05$) between the two types of colorant added sample. The panelists rated the difference between the color and odor of the two samples with the mean values of 2.13±2.67 and 2.33±2.56, respectively. The mode value of difference score equaled 0 indicating that most panelists could not detect the difference between the two colorant added rice balls in coconut milk.

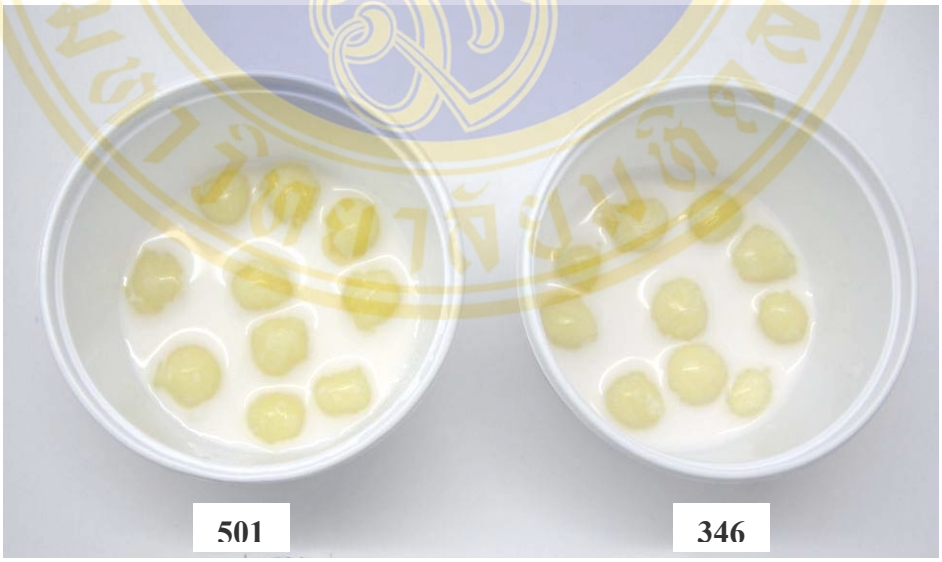

Figure 25 Bua loy num kati with added tartrazine (left) and turmeric colorant (right)

Table 9 Comparison of L*a*b* color parameters of synthesis colorant added and turmeric colorant added Bua loy num kati.

Colorant	CIE L*a*b* color parameter		
	L*	a*	b*
Tartrazine	72.65±0.14[b]	-8.38±0.09[b]	42.45±0.37[a]
Turmeric colorant	71.48±0.28[a]	-9.46±0.24[a]	42.25±0.33[a]

[1] Results are mean ± SD of three batches with triplicate analysis (n=9).
[2] Value in vertical with different superscript (a,b), Paired-sample t t-test was used with significant different ($p \leq 0.05$).

Table 10 Comparison of sensory and difference scores (color and odor) of Bua loy num kati

Evaluation	Sample no.	
	501 (Tartrazine)	346 (Turmeric colorant)
Color acceptance score	7.40±1.10[a]	7.50±1.20[a]
Odor acceptance score	7.03±1.27[a]	7.10±1.16[a]
Color difference score (n=30)		
Mean	2.13±2.67	
Mode	0 (No difference)	
Odor difference score (n=30)		
Mean	2.33±2.56	
Mode	0 (No difference)	

[1] Results are sensory score (mean ± SD) with 30 untrained panelists.
[2] Value in horizontal with different superscript (a,b), Paired-sample t-test was used with significant different ($p \leq 0.05$).
[3] Difference evaluations among pair of sample was evaluated by 15 centimeters long scale to calculate the average of score (mean ± SD) with scale 0=no difference, 0.01-3.75 = slight difference, 3.76-7.50 = moderate difference, 7.51-11.25 = large difference, 11.26-15 = very large difference.

d. Butter cream for cake decoration

Butter cream with tartrazine added had significantly higher ($p \leq 0.05$) L*and b* values than turmeric colorant added sample while its a* value was significant lower ($p \leq 0.05$) (Table 11). Both colorant added samples showed yellowish color. The color of turmeric colorant seemed to fade easily when adding to butter cream indicating a need for a relatively large amount of turmeric colorant in oily food. No problem with precipitation of color occurred in the product. The appearance of both samples of butter cream is shown in Figure 26.

In sensory evaluation (Table 12), the panelists moderately to very much liked the color and odor of turmeric colorant added butter cream with the score being 7.13±1.01 and 7.10±1.24, respectively. They were not significantly different ($p > 0.05$) from tartrazine added butter cream. The mean of color and odor difference scores was 6.17±3.51 and 4.47±3.28, respectively. The mode values were 4 which indicated that most panelists detect a slight difference between the two types of colorant added butter cream.

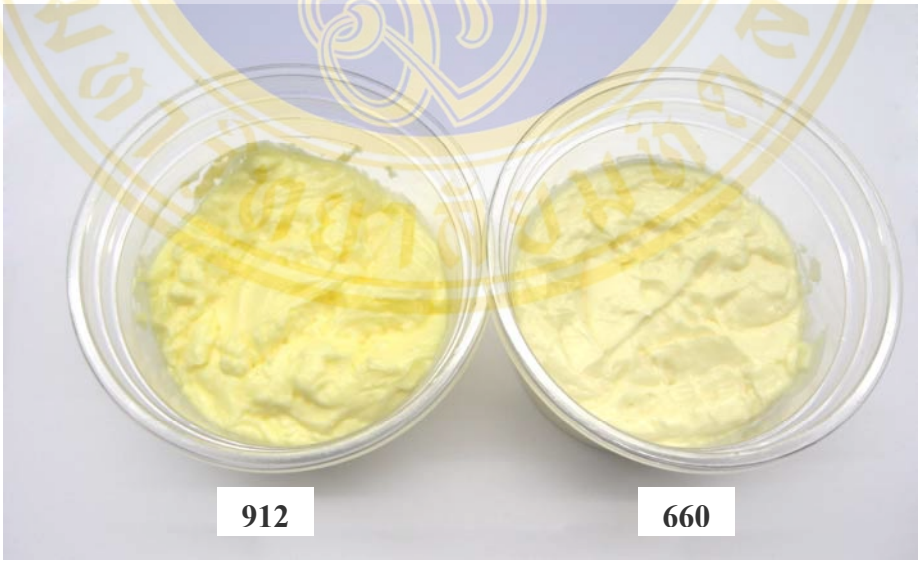

Figure 26 Butter cream with added sunset yellow (left) and turmeric colorant (right)

Table 11 Comparison of L*a*b* color parameters of synthesis colorant added and turmeric colorant added butter cream

Colorant	CIE L*a*b* color parameter		
	L*	a*	b*
Sunset yellow	86.97±0.13 [b]	-7.69±0.03[a]	42.61±0.28[b]
Turmeric colorant	86.64±0.17 [a]	-6.55±0.07[b]	34.35±0.22 [a]

[1] Results are mean ± SD of three batches with triplicate analysis (n=9).
[2] Value in vertical with different superscript (a,b), Paired-sample t t-test was used with significant different ($p \leq 0.05$).

Table 12 Comparison of sensory score and difference evaluation (color and odor) of butter cream

Evaluation	Sample no.	
	912 (Tartrazine)	660 (Turmeric colorant)
Color acceptance score	7.50±1.11[a]	7.13±1.01[a]
Odor acceptance score	7.13±1.04[a]	7.10±1.24[a]
Color difference score (n=30)		
Mean	6.17±3.51	
Mode	4 (Slight difference)	
Odor difference score (n=30)		
Mean	4.47±3.28	
Mode	4 (Slight difference)	

[1] Results are sensory score (mean ± SD) with 30 untrained panelists.
[2] Value in horizontal with different superscript (a,b), Paired-sample t-test was used with significant different ($p \leq 0.05$).
[3] Difference evaluations among pair of sample was evaluated by 15 centimeters long scale to calculate the average of score (mean ± SD) with scale 0=no difference, 0.01-3.75 = slight difference, 3.76-7.50 = moderate difference, 7.51-11.25 = large difference, 11.26-15 = very large difference.

e. Fried rice crispy

The L*and b* values of tartrazine added fried rice crispy were significantly higher ($p \leq 0.05$) than the turmeric colorant added sample while the a* value was significant lower ($p \leq 0.05$) (Table 13). Both of samples showed a light yellow color. At Turmeric colorant was initially added at the amount that could give the same color as the tartrazine added one. Sun drying for a long time (6-8 h), however, caused the color of turmeric colorant added rice crispy to fade and become more pale than synthetic colorant sample. Moreover, deep frying caused color fading in both samples. The appearance of both samples of fried rice crispy is presented in Figure 27.

In sensory evaluation (Table 14), as expected from the above finding the panelists liked the color and odor of tartrazine added rice crispy moderately to very much (7.03±1.50 and 6.30±1.73, respectively). The scores were significantly higher ($p \leq 0.05$) than turmeric colorant added rice crispy (6.40±1.48 and 6.53±1.22, respectively). The panelist rated the difference score between the color of the two samples as 5.67±3.31 with the mode value equaled to 4 indicating a slight difference. The odor difference score was 4.50±3.78 with the mode value being 0 expressing no difference.

Figure 27 Fried rice crispy with sunset yellow (left) and turmeric colorant (right)

Table 13 Comparison of L*a*b* color parameters of synthesis colorant added and turmeric colorant added fried rice crispy.

Colorant	CIE L*a*b* color parameter		
	L*	a*	b*
Tartrazine	72.51±0.04[b]	-3.89±0.29[a]	30.77±1.09[b]
Turmeric colorant	71.08±0.43[a]	-2.87±0.13[b]	21.25±0.80[a]

[1] Results are mean ± SD of three batches with triplicate analysis (n=9).
[2] Value in vertical with different superscript (a,b), Paired-sample t t-test was used with significant different ($p \leq 0.05$).

Table 14 Comparison of sensory score (color and odor) and difference evaluation of fried rice crispy

Evaluation	Sample no.	
	116 (Tartrazine)	102 (Turmeric colorant)
Color acceptance score	7.03±1.50[b]	6.40±1.48[a]
Odor acceptance score	6.30±1.73[a]	6.53±1.22[a]
Color difference score (n=30)		
Mean	5.67±3.31	
Mode	4 (Slight difference)	
Odor difference score (n=30)		
Mean	4.50±3.78	
Mode	0 (No difference)	

[1] Results are sensory score (mean ± SD) with 30 untrained panelists.
[2] Value in horizontal with different superscript (a,b), Paired-sample t-test was used with significant different ($p \leq 0.05$).
[3] Difference evaluations among pair of sample was evaluated by 15 centimeters long scale to calculate the average of score (mean ± SD) with scale 0=no difference, 0.01-3.75 = slight difference, 3.76-7.50 = moderate difference, 7.51-11.25 = large difference, 11.26-15 = very large difference.

f. Orange flavored soft drink

The tartrazine added soft drink gave significantly higher ($p \leq 0.05$) L* and a* values than turmeric colorant added sample while the b* value was lower ($p \leq 0.05$) (Table 15). Both samples contain a consistent amount of sugar, fructose syrup and titanium dioxide but were different in the ratio of yellow color (tartrazine or turmeric colorant) to sunset yellow used. Turmeric colorant dispersed well in water but a thin film occurred on the surface. This film disappeared with added titanium dioxide to soft drink. The appearance of both colorant added soft drinks is shown in Figure 28.

In sensory evaluation (Table 16), the panelists equally preferred the color and odor of both samples ($p > 0.05$). The difference between the color and odor of the two samples was rated at 2.70±7.74 and 4.27±3.71, respectively. The mode values of both sets of scores equaled 0 indicating no difference.

Figure 28 Orange flavored soft drink with sunset yellow (left) and turmeric colorant (right)

Table 15 Comparison of L*a*b* color parameters of synthesis colorant added and turmeric colorant added orange flavored soft drink

Colorant	CIE L*a*b* color parameter		
	L*	a*	b*
Sunset yellow	75.68±0.42[b]	3.87±0.06[b]	38.23±0.13[a]
Turmeric colorant	71.01±0.77[a]	3.30±0.09[a]	40.68±0.44[b]

[1] Results are mean ± SD of three batches with triplicate analysis (n=9)
[2] Value in vertical with different superscript (a,b), Paired-sample t t-test was used with significant different ($p \leq 0.05$)

Table 16 Comparison of sensory score and difference evaluation (color and odor) of orange flavored soft drink

Evaluation	Sample no.	
	052 (Tartrazine)	246 (Turmeric colorant)
Color acceptance score	7.17±1.32[a]	6.93±1.46[a]
Odor acceptance score	7.37±1.07[a]	7.07±1.46[a]
Color difference score (n=30)		
Mean		2.70±7.74
Mode		0 (No difference)
Odor difference score (n=30)		
Mean		4.27±3.71
Mode		0 (No difference)

[1] Results are sensory score (mean ± SD) with 30 untrained panelists.
[2] Value in horizontal with different superscript (a,b), Paired-sample t-test was used with significant different ($p \leq 0.05$).
[3] Difference evaluations among pair of sample was evaluated by 15 centimeters long scale to calculate the average of score (mean ± SD) with scale 0=no difference, 0.01-3.75 = slight difference, 3.76-7.50 = moderate difference, 7.51-11.25 = large difference, 11.26-15 = very large difference.

g. Orange flavored jelly

Both samples contained a mixture of yellow color (tartrazine or turmeric colorant) and sunset yellow. Turmeric colorant added jelly showed significantly higher ($p \leq 0.05$) L*and b* values than tartrazine added jelly while the a* value was lower ($p \leq 0.05$) (Table 17). The appearance of both colorant orange flavored jelly is shown in Figure 29.

In sensory evaluation (Table 18), the panelists significantly preferred the color of turmeric colorant added jelly with the score of 7.57±1.01 (like moderately to very much. The acceptance for odor was not significantly different ($p > 0.05$). The difference between the color and odor was 4.83±3.79 and 5.07±3.84, respectively. The mode value of both scores equaled 4 indicating that most panelists detected a slight difference in color and odor between the two colorant added samples.

Figure 29 Orange flavored jelly with sunset yellow (left) and turmeric colorant (right)

Table 17 Comparison of L*a*b* color parameters of synthesis colorant added and turmeric colorant added orange flavored jelly.

Colorant	CIE L*a*b* color parameter		
	L*	a*	b*
Tartrazine	31.32±0.75[a]	1.91±0.14[b]	18.17±0.55[a]
Turmeric colorant	34.01±0.78[b]	1.42±0.18[a]	21.29±1.39[b]

[1] Results are mean ± SD of three batches with triplicate analysis (n=9).
[2] Value in vertical with different superscript (a,b), Paired-sample t t-test was used with significant different ($p \leq 0.05$).

Table 18 Comparison of sensory score and difference evaluation (color and odor) of orange flavored jelly

Evaluation	Sample no.	
	025 (Tartrazine)	726 (Turmeric colorant)
Color acceptance score	7.10±1.06[a]	7.57±1.01[b]
Odor acceptance score	6.47±1.57[a]	6.93±1.62[a]
Color difference score (n=30)		
Mean	4.83±3.79	
Mode	4 (Slight difference)	
Odor difference score (n=30)		
Mean	5.07±3.84	
Mode	4 (Slight difference)	

[1] Results are sensory score (mean ± SD) with 30 untrained panelists.
[2] Value in horizontal with different superscript (a,b), Paired-sample t-test was used with significant different ($p \leq 0.05$).
[3] Difference evaluations among pair of sample was evaluated by 15 centimeters long scale to calculate the average of score (mean ± SD) with scale 0=no difference, 0.01-3.75 = slight difference, 3.76-7.50 = moderate difference, 7.51-11.25 = large difference, 11.26-15 = very large difference.

h. Orange flavored ice confection

The L* value of tartrazine and turmeric colorant added ice confection did not significantly differ ($p>0.05$) whereas the a* value of the former and b* value of the latter was significantly higher ($p\leq0.05$) (Table 19). Turmeric colorant gave a more consistent shade of color after freezing of this product while the tartrazine added ice confection appeared less yellowish after freezing. The appearance of both colorant added orange flavoured ice confection is shown in Figure 30.

In sensory evaluation (Table 20), the panelists equally preferred ($p>0.05$) the color and odor of both samples. The mean difference score between the color and odor of the two samples was 4.13±3.86 and 2.70±2.55, respectively. The mode value of color difference score was 4 and of odor difference score was 0 indicating that most panelists detected a slight difference in color but no difference in odor between the two colorant added samples.

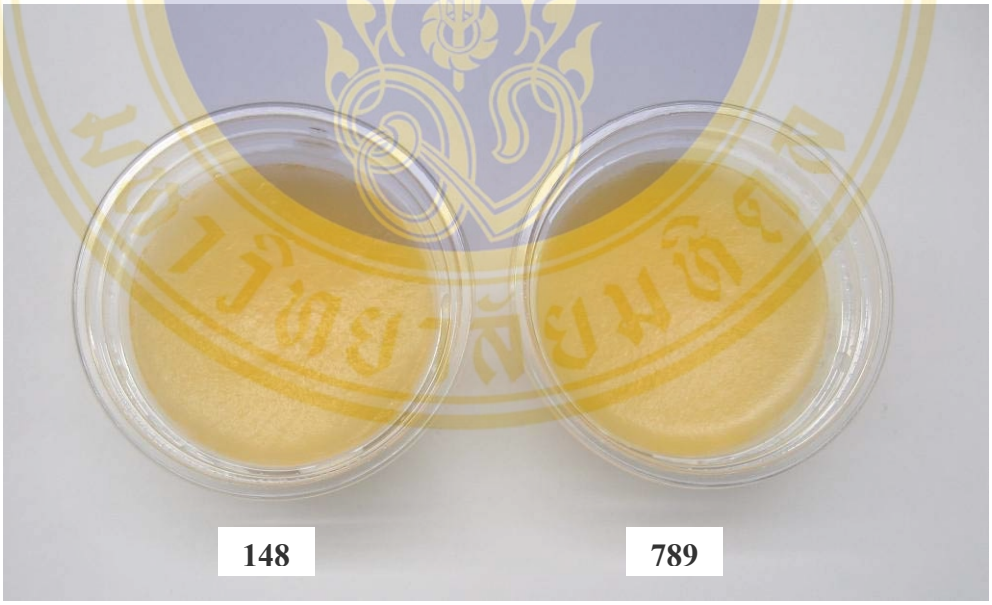

Figure 30 Orange flavored ice confection with tartrazine (left) and turmeric colorant (right)

Table 19 Comparison of L*a*b* color parameters of added synthesis colorant and turmeric colorant added orange flavored ice confection

Colorant	CIE L*a*b* color parameter		
	L*	a*	b*
Tartrazine	62.14±3.52[a]	4.75±1.12[a]	29.24±4.69[a]
Turmeric colorant	60.56±1.56[a]	3.05±0.54[b]	32.99±1.53[b]

[1] Results are mean ± SD of three batches with triplicate analysis (n=9).
[2] Value in vertical with different superscript (a,b), Paired-sample t t-test was used with significant different ($p \leq 0.05$).

Table 20 Comparison of sensory score and difference evaluation (color and odor) of orange flavored ice confection

Evaluation	Sample no.	
	148 (Tartrazine)	789 (Turmeric colorant)
Color acceptance score	6.97±1.56[a]	7.10±1.30[a]
Odor acceptance score	7.10±1.30[a]	7.30±1.27[a]
Color difference score		
Mean		4.03±3.74
Mode		4 (Slight difference)
Odor difference score		
Mean		2.68±2.49
Mode		0 (No difference)

[1] Results are sensory score (mean ± SD) with 30 untrained panelists.
[2] Value in horizontal with different superscript (a,b), Paired-sample t-test was used with significant different ($p \leq 0.05$).
[3] Difference evaluations among pair of sample was evaluated by 15 centimeters long scale to calculate the average of score (mean ± SD) with scale 0=no difference, 0.01-3.75 = slight difference, 3.76-7.50 = moderate difference, 7.51-11.25 = large difference, 11.26-15 = very large difference

CHAPTER VI
DISCUSSION

6.1 Turmeric pomace

Fresh turmeric pomace, obtained from a pharmaceutical industry, had a high moisture content from water which is used as a solvent for extracting active compounds for pharmaceutical purposes. According to the manufacturer, extraction is performed by boiling in water. Then the mixture is drained and the pomace discarded as a by-product. After receiving the fresh pomace from the industry, removing the excess water was necessary to make the raw material easy to store. The color of fresh turmeric pomace was dark orange because of the curcuminoid compound and the brown pigment from enzymatic browning that occurred during processing, handling and transportation (41). After excess water separation, turmeric pulp still contained 71.71±0.22 %w/w water according to the study of Lertphatcharanon S (78).

6.2 Carotenoids content in turmeric by-product

Although turmeric by-product had yellowish color that was suspected to partly come from carotenoids, the analytical result indicated low carotenoids content (17.78 μg/100g) (Appendix D). Kandlakunta B (79) reported that turmeric had small amount of carotenoids (510 ± 0.11μg/100g). This might explain the much lower level of carotenoids in turmeric by-product. Consequently, extraction, determination and storage study of these compounds in the turmeric colorant extracted was omitted.

6.3 Extraction of curcuminoids from turmeric pulp to produce turmeric colorant

6.3.1 Effect of evaporation for turmeric colorant extraction

The ethanolic extraction from turmeric pulp was evaporated to make a colorant with five-fold concentration. In the first experiment, evaporation to get a ten-fold concentration was attempted. The concentrate was deep red brown and highly viscous. This may be due to occurrence of turmeric oleoresin as described in the flow chart of turmeric product preparation as Figure 31. Oleoresin is a product gained from desolventing after organic solvent extraction. Although turmeric oleoresin can be used as colorant in foodstuff industry, it is difficult to handle at room temperature and use in household cooking that does not use nonvolatile edible diluents or emulsifiers such as propylene glycol used in food industry (41). To produce colorant in curcumin powder form, oleoresin crystallization is carried out in the last step of flow chart below. However, curcumin powder is not approved by the USFDA for general use (36). Therefore, evaporation was performed to obtain five-fold concentration and a turmeric colorant liquid form that is soluble and easily to apply in home cooking with less ethanol retained.

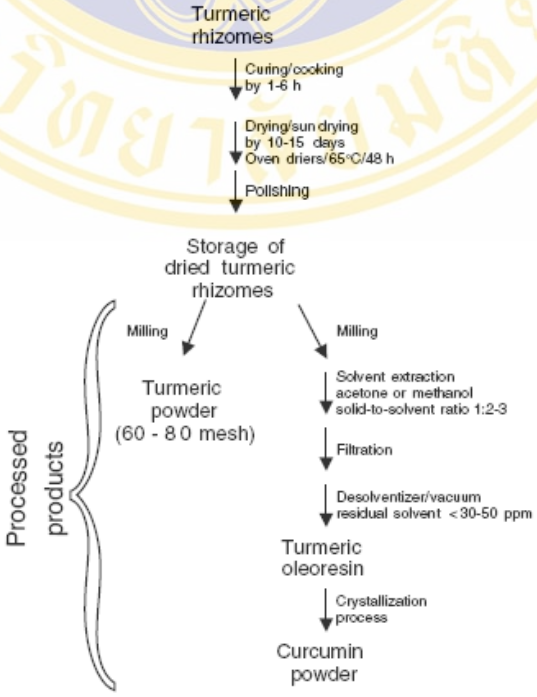

Figure 31 Preparation of turmeric product (31)

6.3.2 Yield of extraction

The yield of curcuminoids in turmeric colorant prepared in this study was 53%. The concentration was lower than other published reports such as Dandekar DV *et al.* (80) that extracted curcuminoids in acetone by using microwave assisted extraction technique to get the yield of pigment 7.98 wt%. However, inedible solvent was used in their study. The loss of curcuminoid may occur from heat and light degradation during post harvest and essential oil production (41, 81).

Although the study found low pigment content from ethanolic extraction, Pothitirat W suggested ethanolic extraction before using Soxhlet apparatus was an appropriate method to extract pigment when comparing the yield of pigment with cost of extraction and time used (44). Moreover, the low yield in this study may occur not only because of extraction method but also the fact that turmeric by-product may have lost a substantial amount of curcuminoids during industrial process. Particle size of turmeric pulp used for extraction, solvent used and thermal process also affected yield of extraction (41).

6.3.3 Appearance of turmeric colorant extraction

Turmeric colorant showed a dark orange to brown color depending upon the volume and the corresponding intensity of mixed curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin). The color also varied with the type of solvent (41). Turmeric colorant is water insoluble but could be dispersed in water. Furthermore, it is soluble in polar solvent such as methanol, ethanol (33).

6.4 Stability study of turmeric colorant

The storage of turmeric colorant was undertaken and its curcuminoid concentration by HPLC, antioxidant activity (FRAP and DPPH) and L*a*b* color parameters were followed.

6.4.1 Temperature effect

Temperature had little or no effects on the curcuminoid concentration, antioxidant capacity and CIE L*a*b* values of turmeric colorant during the 2-month

storage. Storage at either 4 or room temperature produced a slight loss of curcuminoids. A higher temperature range such as that bused in food preparation and processing may result in a greater loss of the compounds. The loss of active principles in spices in cooked food was observed by Suresh D *et al*. They found curcumin loss from heat processing of turmeric was 27-53% with a maximum loss in pressure cooking (25).

Different storage temperature (4°C and room temperature) showed negligible effect on both the DPPH and FRAP antioxidant capacity. This was contradictory to the result reported by Jang H-D *et al.* (22). They found the antioxidant capacity of DPPH and FRAP after one-month storage of turmeric extract at 4°C was higher than room temperature. For CIE L*a*b* color parameter, increasing temperature caused higher L*a*b* values with significant difference. The colorant solution, however, appeared similar visually even with the parameter change throughout storage.

6.4.2 Protective agent effect

Antioxidant, dl-α- tocopherol, was added to turmeric colorant to test the effect of a protective agent on storage of colorant. The changes in curcuminoid concentration, antioxidant activity (FRAP and DPPH) and L*a*b* color parameters tended to be similar in the samples with or without dl-α- tocopherol.

Generally, the antioxidant activity of curcuminoids is comparable to vitamin C and E. Therefore they, themselves are prone to being oxidized. Oxidation of these compounds can be prevented by protection from sunlight and addition of other antioxidants or protective agents (36, 52). This study selected to use dl-α-tocopherol, a commonly used antioxidant in food products because of its ability to dissolve in organic solvents, similar to curcuminoids. Nonetheless the use of dl-α-tocopherol did not improve the stability of curcuminoids during the 2-month storage in this study.

6.5 Curcuminoid concentration of storaged turmeric colorant

Curcuminoids content in turmeric colorant were found to be fluctuating with a small overall change during the 2-month storage under any of the storage conditions. This result did not agree with the result published by Wang Y-J *et al.* that presented the first-order plots for the degradation of curcumin at various pH values in two hours (24). There is no information about curcuminoids stability in long term study. Nevertheless, it may be assumed based on the antioxidant mechanism of curcuminoids proposed by Masuda T *et al.* Curcuminoids antioxidation process may be reversible and likely to occur in the first stage of nonenzymatic antioxidant process leading to a fluctuating trend of curcuminoid concentration (56).

6.6 Antioxidant capacity of turmeric colorant

DPPH capacity of turmeric colorant was 3.04 ± 0.19 mmol/ 100ml while the value for fresh turmeric by-product was 4.43 ± 0.24 mmol TE/100 g. Similarly, its FRAP capacity was 2.92 ± 0.17 mmol/ 100ml decreasing from 7.76 ± 0.51 mmol TE/100 g in fresh turmeric by-product. Antioxidant capacity of turmeric colorant was decreased because degradation of curcuminoids occurred during the preparation process of colorant.

DPPH value of turmeric colorant in this study could be considered relatively high according to the report by Kevers C *et al.* They found the highest DPPH value of fruits and vegetable in their study from strawberry and yellow pepper, 0.68±0.04 and 1.20±0.12 mmol TE/100 g fresh weight, respectively (82). It should be noted, however, that values from different studies should not directly be compared due to the possible discrepancies in the analytical procedure.

Average TEAC value of turmeric colorant by DPPH and FRAP assay at the beginning of study was 3.04± 0.24 and 2.92±0.17 mmol TE/100 ml, respectively. Both of TEAC values were relatively high when compared with those from another study of Wojdylo A *et al.* (83) They found TEAC values of DPPH and FRAP were 0.100±0.002 and 0.626±0.001mmol TE/100 g, respectively in dried turmeric. The high TEAC level in this study may be explained by the fact that turmeric colorant was

concentrated 5 time from the fresh turmeric ethanolic extract. Furthermore, their study used freeze dried form of spice powder extract. Wojdylo A *et al.* (83) also divided fruits and vegetable into 5 groups: (a) very low FRAP: <10 μM/100 g, (b) low FRAP: 10–50 μM/100 g, (c) good FRAP: 50– 100 μM/100 g, (d) high FRAP: 100–500 μM/100 g and (e) very high FRAP: >500 μM/100 g. Hence, turmeric colorant could be in a group of "very high FRAP" according to their classification. Another study by Katalinic V *et al.* divided the FRAP levels using different range. Turmeric colorant also was in their "very high" group (84).

During the 2-month storage, the antioxidant capacity of turmeric colorant showed a fluctuating trend. This behavior was also found in various studies of Kevers C *et al.* (82), Chou S-T *et al.* (85) and Yang J *et al.*(86). After two months the antioxidant capacity was still relatively high when compared with the result of Wojdylo A *et al.* (83).

The antioxidant capacity of turmeric colorant at 0 day was confirmed by ORAC assay. The value was 76.9 mmol/100 ml. This ORAC value was comparatively high for other studies such as Kevers C *et al.* , Wu X *et al.* and Cz M *et al.* Three other reports on the ORAC value of various vegetables and fruits also found no ORAC value in any samples higher than that of turmeric colorant. (82, 87, 88)

6.7 CIE L*a*b color parameters of turmeric colorant

6.7.1 Effect of type of solvent

Turmeric colorant was dissolved in two solvents, ethanol and deionized water. The L*a*b* color parameters varied with the type of solvent. Turmeric colorant was soluble in organic solvent as ethanol to give extremely bright color with high L* value. It could be dispersed in deionized water in which it appeared more turbid than the former. Many studies revealed that turmeric colorant was insoluble in water (15, 40, 41). Nevertheless, the turmeric colorant prepared this study could be dispersed in water and no separation was observed for 2-3 days.

6.7.2 Effect of pH

Turmeric colorant was dissolved in various solutions from pH4 to pH10. It gave a yellowish color in acidic but orange yellowish in basic condition. The study by Goel A *et al.* displayed the full range of color change for turmeric in neutral, acidic and basic conditions (15). Different pH condition may affect curcuminoid degradation. Price LC *et al.* (23) reported the degradation rate of curcuminoids in alkaline solution while Wang Y-J *et al.* found degradation product such as vanillin, ferulic acid and feruloyl methane (24). Part of feruloyl methane can participate in condensation reactions to give yellow-brownish color compounds (41).

Although turmeric colorant could change the color in basic condition, foods with a basic pH are not common. Hence, application of turmeric colorant in food products at different pH would not be hindered by this effect.

6.8 Application of turmeric colorant in food products

Turmeric colorant was applied in 8 different types of food to determine the color value, appearance and consumer acceptance. Turmeric colorant was found to be tolerant of common food preparation conditions such as long mixing time (30 min), steaming (15 min) and cooking in boiling water. The results agreed well with the study of Sowbhagya HB *et al.* (26) in which they applied curcumin in extruded product to compare with tartrazine. Although the color change was more marked in the extrudate containing curcumin compared to tartrazine, the author concluded that curcumin showed a good potential for such use. Furthermore, use of turmeric in several household recipes has been a long tradition in various countries in Asia. Sun drying (like in the preparation of crispy rice) caused the greatest problem of color fading. The main reason was oxidation of the color compounds during a long exposure to sunlight (36). This problem could be solved with adding more turmeric colorant along with other spices in the recipe such as garlic and pepper to mask the turmeric scent.

Turmeric colorant showed a bright yellowish color when mixed with ingredients like starch or flour whereas it appeared to be faded when mixed with a large portion of oil and fat such as in butter cream. Adding turmeric colorant in oily foods, may, therefore need a larger amount of colorant to ensure the desired color intensity. This may in turn affect the food odor due to essential oils that were retained

in turmeric colorant (89). The effect to the food quality and consumer acceptability could be either positive or negative depending on the type of food. Moreover, other flavoring compounds may be added to lessen the problem.

Turmeric colorant was found quite suitable as a coloring agent in beverages and related products. In this study, turmeric colorant could be used instead of tartrazine (an artificial colorant) with good consumer acceptance. Calvo C *et al*. (27) published the curcumin can replace the artificial color used commercially to color jellies with similar visually. The amount of turmeric colorant that is used in food differed among the types of food. It was noted that turmeric colorant worked well in orange flavored drink, jelly and ice confection. It even surpassed tartrazine in term of color quality in ice confection which underwent a freezing process. After freezing, turmeric colorant added sample gave an orange yellowish color while tartrazine added sample appeared reddish orange, different from the expected orange color. Hence, turmeric colorant could be a suitable alternative for frozen products since it would be easy to predict the color of the sweet liquid after freezing.

A possible drawback in the application of turmeric colorant in food products could be the strong scent of its essential oil. Nonetheless the color as well as odor quality of all foods tested in this study were accepted by the panelists. The mean sensory scores (from 9-point hedonic scales) were between like moderately to very much. Furthermore, turmeric tended to give a synergistic effect to the orange flavor in the orange flavored drink, jelly and ice confection.

Overall, the results indicated that turmeric colorant had a good potential for application in several common food products including Thai traditional desserts. It could be used as an alternative to artificial color. Besides, turmeric color with its curcuminoid content may help to contribute some antioxidant capacity to food.

CHAPTER VII
CONCLUSION

Turmeric by-product can be extracted with 95% ethanol at a ratio of 1:5 (kg wet weight turmeric pulp: liter of alcohol) to prepare turmeric colorant. The colorant could be produced with a five-fold concentration of the extract which was suitable for use in food instead of an artificial color. Whereas a ten-fold concentrate was too dark in color, highly viscous and difficult to disperse.

The turmeric colorant and contained at least three types of curcuminoid compounds (curcumin, demethoxycurcumin, bis-demethoxycurcumin). The turmeric colorant showed relatively high DPPH and FRAP antioxidant capacity. The colorant could be stored for two months at either 4ºC or room temperature, with or without a protective agent (dl-α-tocopherol), with only a slight change in its color curcuminoid content and antioxidant capacity.

Turmeric colorant could provide different shades of yellow color depending upon the type of solvent and pH (pH 4-10). Turmeric colorant dissolved in 95% ethanol showed a brighter yellow color than when dissolved in deionized water. In this study, turmeric colorant showed a yellowish color in acidic condition but changed to a red color in basic condition.

Turmeric colorant can be used in various foods such as dessert and beverage. It could be used instead of tartrazine, a synthetic colorant. The colorant exhibited a good stability with heat (steam or boiling) and cold temperature (freezing) during food preparation. Turmeric colorant dissolved readily in oil but a larger amount was required when added to oily foods. Although turmeric colorant was water insoluble, it could disperse in water and remained dispersed for 2-3 days. It also enhanced the orange flavor in some of the food product tested. On the contrary, flavoring compounds may be needed in certain products to mask the scent of essential oils in turmeric.

The use of turmeric by-product from pharmaceutical industry as a source of natural food colors may be a reasonable alternative to reduce a disposal problem and add more value to the raw material. Moreover, further research could be undertaken to investigate the microbial, pesticide and heavy metal contamination in turmeric colorant to confirm consumer safety. Its relatively high antioxidant capacity is another interesting property that should be followed longer than two months. Curcuminoids content and antioxidant capacity per serving of cooked or processed food could also be an interesting research topic in future.

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APPENDICES

APPENDIX A
CURCUMINOIDS ANALYSIS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

The method used was a modified method of Boonchong P and Saohin W (19).

Chemicals

- Standard curcumin (mixture of curcumin, demethoxycurcumin and bisdemethoxycurcumin, Fluka, 28260)
- Acetonitrile
- Glacial acetic acid
- 95% Ethanol

Equipments

- HPLC
- Analytical balance (Mettler Toledo, AG 204)
- Whatman syringe filter (Nylon, 0.2 μm)

Reagent preparation

Mobile phase, 1000 ml

Mobile phase consist of 1% of acetic acid and acetonitrile at a ratio of 45:55. In 1000 ml of mobile phase, 450 ml of 1% acetic acid was mixed with 550 ml acetonitrile then pass through the 0.45 μm Whatman filter membrane. The reagent was kept in amber glass bottle until performed HPLC.

1% acetic acid, 1000 ml

10 ml of glacial acetic acid was diluted with deionized water to adjust 1000 ml in volumetric flash. A solvent was kept in amber glass bottle until used to prepare mobile phase.

HPLC condition

- Waters 510 HPLC pump
- Mobile phase : 1% acetic acid and acetonitrile (45:55 v/v)
- C_{18} column (250 x 4.6 mm, 5µm particle size, Econosphere)
- Injection volume: 20 µl
- Flow rate: 1 ml/min
- Hewlett Packard series 1050 UV-Visible detector
- Optimum wavelength 425 nm

Calculation of total curcuminoid concentration

Total peak area = peak area 1 + peak area 2 + peak area 3

[1] curcumin

[2] demethoxycurcumin

[3] bisdemethoxycurcumin

Total peak area was compared with standard curve to calculate the curcuminoid concentration (µg/ml) using the following equation.

$$\text{Total curcuminoid concentration (mg/ml)} = \frac{\text{Curcuminoid conc. from standard curve} \times \text{volume (ml)} \times \text{dilution factor}}{\text{sample (g)} \times 1000}$$

APPENDIX B
METHOD OF DPPH FREE RADICAL SCAVENGING ASSAY

The method used was a modified method of Burda S and Oleszek W (75).

Chemicals

- 2,2-diphenyl-1-pierythydrazyl (DPPH, Sigma Aldrich, USA, MW 394.32)
- Trolox (6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, 97%, Sigma Aldrich, Germany)
- Butylated hydroxyanisole (BHA, Sigma Aldrich, USA)
- 95% Ethanol

Equipment

Analytical balance (Mettler Toledo, AG 204)
Vortex
Spectrophotometer

Chemical solution preparation

0.15 mM DPPH solution: DPPH 6 milligrams was dissolved with 95%Ethanol and adjusted to 100 ml in volumetric flask. DPPH solution was filtered through Whatman filter No.2 before use in analysis.

1.0 mg/ml BHA solution: Ten milligrams of BHA was weighed and dissolved with a little of ethanol, then adjusted to 10 ml by deionized water in a volumetric flask.

Standard trolox solution: Trolox 50.1 milligrams were precisely weighed. Then, Trolox was dissolved with 95%Ethanol and adjusted to 25 ml. The final concentration was 8 mM. Before analysis, standard trolox solution was prepared in range of concentration 0.005, 0.01, 0.02, 0.04, 0.06, 0.08 mM.

Procedure

1. Add 1 ml of supernatant of turmeric fresh extraction or turmeric colorant into each tube as shown in table ….

Amount of solution for DPPH assay in each tube

Tube	Consist of
Standard	Trolox 1 ml + DPPH solution 2 ml
Blank	Sample 1 ml + 95%Ethanol 2 ml
Sample	Sample 1 ml + DPPH solution 2 ml (Sample: 1 ml of fresh turmeric extraction or turmeric colorant)
Positive control	BHA 1 ml + DPPH solution 2 ml
Negative control	Deionized water 1 ml + DPPH solution 2 ml

2. Placed the tube in the dark at room temperature for exactly 30 minutes
3. The absorbance is measured at 517 nm using a spectrophotometer.
4. Absorbance data of trolox ethanol solution tubes are used for standard curve.
5. The absorbance data of sample is recorded and used trolox standard curve to calculate the DPPH activity.

APPENDIX C

Method of ferric-reducing antioxidant power assay (FRAP)

The method used was a modified method of Benzie IFF and Strain JJ (76).

Chemicals

- Sodium acetate hydrated ($CH_3COONa.3H_2O$, Ajax Finechem, Australia)
- Glacial acetic acid (J.T Beaker, USA)
- Hydrochloric acid (J.T Beaker, USA)
- Ferric chloride hexahydrate ($FeCl_3.6H_2O$)
- 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid 97% (Trolox) (Sigma Aldrich, Germany)
- 2,4,6-Tris(2-pyridyl)-1,3,5-triazine (TPTZ, Fluka, 93285)

Equipment

Analytical balance (Mettler Toledo, AG 204)
Vortex
Spectrophotometer

Chemical solution preparation

FRAP reagent: was freshly prepared by mixing 300 mM acetate buffer, 10 mM TPTZ and 20 mM ferric chloride with ratio of 10:1:1 (v/v/v) and kept in amber glass.

300 mM acetate buffer, pH 3.6, 1 Liter: weigh 3.1 g Sodium acetate hydrated with added 16 ml glacial acetic acid, dissolved all with 900 ml deionized water. The solution was adjusted to pH 3.6 by acetic acid or sodium hydroxide and adjusted to 1000 ml with deionized water. This solution was kept at 4°C.

10 mM TPTZ, 100 ml: 0.31g TPTZ was dissolved with 40 mM HCl 100 ml in water bath at 50°C. This solution stable for 1 week with kept at 4°C.

20 mM ferric chloride, 100 ml: 0.5406 g $FeCl_3.6H_2O$ was dissolved with 100 ml deionized water. This solution stable for 1 week with kept at 4°C.

40 mM HCl, 100 ml: Concentrated HCl 0.33 ml was pipetted and adjusted volume with deionized water to 100 ml. This solution was kept at room temperature.

40 mM HCl, 100 ml: Concentrated HCl 0.33 ml was pipetted and adjusted volume with deionized water to 100 ml. This solution was kept at room temperature.

Standard trolox solution

Trolox 1 ml of 1000 µM trolox stock solution was pipetted and diluted with 9 ml deionized water to gain 100 µM trolox working solution. For analysis, standard trolox solution was prepared serial dilution in range of concentration 100, 50, 25, 12.5, 6.25 µM trolox standard solution.

Procedure

1. Add 1 ml of supernatant of turmeric fresh extraction or turmeric colorant into each tube as shown in table ….

Amount of solution for FRAP assay in each tube

Tube	Consist of
Standard	Trolox 1 ml + FRAP reagent 3 ml
Blank	Sample 1 ml + Deionized water 3 ml
Sample	Sample 1 ml + FRAP reagent 3 ml (Sample: 1 ml of fresh turmeric extraction or turmeric colorant)
Control	Deionized water 1 ml + FRAP reagent 3 ml

2. The solution was mixed and incubated in the dark at 37°C for exactly 4 minutes
3. The absorbance is measured at 593 nm using a spectrophotometer.
4. Absorbance data of trolox solution tubes are used for standard curve.
5. The absorbance data of sample is recorded and used trolox standard curve to calculate the FRAP activity.

APPENDIX D

Carotenoid content result from Institute of Nutrition Laboratory

INMU Service

FOOD AND NUTRITION TECHNICAL SERVICES
INSTITUTE OF NUTRITION, MAHIDOL, UNIVERSITY
Salaya, Putthamonthon, Nakhonpathom 73170, Thailand

ตัวอย่างอาหาร : กากขมิ้นชัน

เลขที่บริการ : SFC 1722/2551

รายละเอียดของตัวอย่างอาหาร : เนื้อละเอียดสีเหลือง บรรจุขวดพลาสติก จำนวน 3 ขวด (ไม่มีฉลาก)

ผู้ขอรับบริการ : โครงการวิจัย การสกัด จำแนก และศึกษาสมบัติการต้านออกซิเดชั่นของสารให้สีจาก ส่วนเหลือทิ้งของอุตสาหกรรมสมุนไพรขมิ้นชัน สถาบันวิจัยโภชนาการ มหาวิทยาลัยมหิดล

วันที่รับตัวอย่าง : 23 เมษายน 2551

ผลการตรวจสอบ/วิเคราะห์ : (ต่อ 100 กรัม)

β-carotene (µg)	17.78

(ผู้ช่วยศาสตราจารย์สมเกียรติ โกศัลวัฒน์)
หัวหน้าฝ่ายเคมีทางอาหาร

(รองศาสตราจารย์พงศธร สังข์เผือก)
รองผู้อำนวยการฝ่ายบริหาร ปฏิบัติราชการแทน
ผู้อำนวยการสถาบันวิจัยโภชนาการ

รายงานผลการวิเคราะห์ตามหนังสือเลขที่ ศธ 0517.21/ 1074 ลงวันที่ 3 มิถุนายน 2551

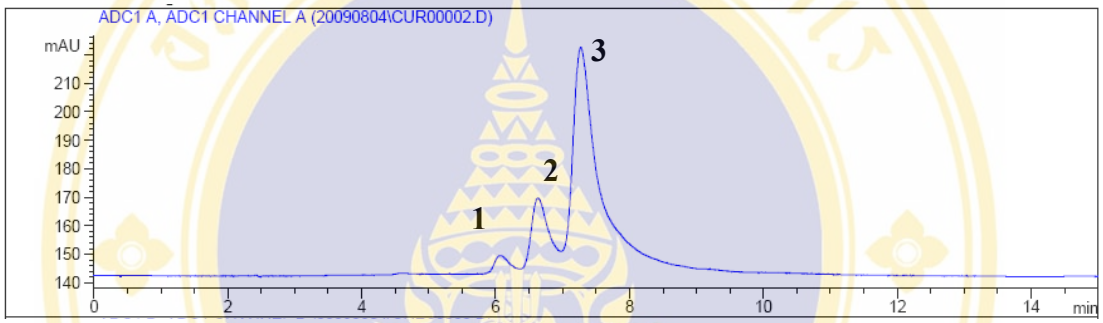
1 / 1

งานบริการวิชาการ สถาบันวิจัยโภชนาการ มหาวิทยาลัยมหิดล ศาลายา พุทธมณฑล นครปฐม 73170
Tel. 02 441 9346, 02 800 2380 ext. 406, 418; Fax. 02 441 9344

APPENDIX E

Chromatogram of standard curcuminoid

The example of chromatogram of standard curcuminoids at concentration 10µg/ml



1 = bisdemethoxycurcumin

2 = demethoxycurcumin

3 = curcumin

APPENDIX F

Ingredient of eight food sample

a. Glossy sticky rice - Thai dessert

Sticky rice	¾ cups
Coconut milk	70 grams
Sugar	75 grams

b. Kanom Num Dok Mai (rice cake) - Thai dessert

Rice starch	1 ¼ cups
Rough starch	1 table spoon
Sugar	½ cups
Drinking water	1 cup
Jasmine extract flavor	10 drops of glass dropper

c. Bua loy num kati (Thai rice balls in coconut milk) - Thai dessert

Sticky rice	1 cup
Drinking water	1 cup
Coconut milk	2 cups
Sugar	1 cup
Salt	1 tea spoon

d. Butter cream cake

Butter	125 grams
Shortening	125 grams
Sugar	280 grams
Drinking water	140 grams

e. Fried rice crispy

Tapioca starch	3 cups
Salt	1 tea spoon
Grinded garlic	1 tea spoon
Grinded pepper	1 tea spoon
Boiling water	2 cups

f. Orange flavored soft drink

Fructose	30 milliliters
Sugar	30 grams
Citric acid	2 gram
Titanium dioxide	15 milligrams
Drinking water	500 milliliters
Orange extract	500 micrograms
Sunset yellow (5mg/ml)	1 milliliter
Tartrazine (5mg/ml)	300 microliters

g. Orange flavored jelly

Fructose	30 milliliters
Sugar	30 grams
Citric acid	500 milligrams
Agar	7 grams
Drinking water	500 milliliters
Orange extract	500 micrograms
Sunset yellow (5mg/ml)	1 milliliter
Tartrazine (5mg/ml)	300 microliters

h. Orange flavored ice confection

Same formula as orange flavored soft drink but storage at -20°C

APPENDIX G

แบบประเมินความพอใจผลิตภัณฑ์แต่งสีจากธรรมชาติ

ผู้ประเมิน ชื่อ..... เพศ ☐หญิง ☐ชาย อายุ.....ปี วันที่..... เวลา .....น.

ข้อแนะนำ: ท่านได้ถูกนำเสนอตัวอย่างผลิตภัณฑ์.....โปรดประเมินตัวอย่างที่ให้ต่อไปนี้โดยการสังเกตุสีและดมกลิ่นเท่านั้น (ไม่ต้องรับประทาน) และตรวจสอบว่าท่านชอบ/ไม่ชอบมากเพียงไรในผลิตภัณฑ์ ใช้สเกลที่เหมาะสมในการแสดงทัศนคติของท่านโดยทำเครื่องหมาย ✕ ในช่องสเกลที่อธิบายความรู้สึกของท่านได้ดีที่สุด

สเกล	*สีของตัวอย่าง*		*กลิ่นของตัวอย่าง*	
	821	582	821	582
ชอบมากที่สุด				
ชอบมาก				
ชอบปานกลาง				
ชอบเล็กน้อย				
เฉยๆ				
ไม่ชอบเล็กน้อย				
ไม่ชอบปานกลาง				
ไม่ชอบมาก				
ไม่ชอบมากที่สุด				

ท่านได้รับตัวอย่างผลิตภัณฑ์ 2 ตัวอย่าง เพื่อเปรียบเทียบในลักษณะสีและกลิ่น กรุณาตรวจสอบจุดต่างๆบนเส้นตรงและทำเครื่องหมาย ✕ ตรงจุดที่สามารถอธิบายลักษณะความแตกต่างของตัวอย่างทั้งสอง ได้ดีที่สุด

ความแตกต่างของสีภายในคู่ทดสอบ

ไม่แตกต่าง แตกต่างเล็กน้อย แตกต่างปานกลาง แตกต่างมาก แตกต่างมากที่สุด

ความแตกต่างของกลิ่นภายในคู่ทดสอบ

ไม่แตกต่าง แตกต่างเล็กน้อย แตกต่างปานกลาง แตกต่างมาก แตกต่างมากที่สุด

ข้อเสนอแนะ

.....

APPENDIX H

Curcuminoid concentration of turmeric colorant at different time and storage conditions

Storage time	Curcuminoid concentration (mg/ml)		
	4ºC	room temperature	room temperature + dl-α-Tocopherol
0 day	10.02±1.04^{a,A}	9.03±1.33^{a,A}	10.75±0.63^{c,A}
3 days	7.41±1.07^{a,A}	7.83±0.90^{a,A}	7.88±0.31^{a,A}
1 week	8.66±0.39^{a,A}	7.82±0.58^{a,A}	8.41±0.26^{ab,A}
2 weeks	7.81±0.86^{a,A}	7.59±1.21^{a,A}	8.07±1.28^{a,A}
4 weeks	8.48±0.50^{a,A}	8.76±1.27^{a,A}	8.67±0.67^{ab,A}
6 weeks	8.46±0.67^{a,A}	9.19±0.55^{a,A}	9.50±0.74^{bc,A}
8 weeks	8.20±1.57^{a,A}	9.00±0.90^{a,A}	8.84±0.65^{ab,A}

[1] Value in vertical with different superscript (a,b), one way ANOVA followed by the Duncan test was used with significant different ($p \leq 0.05$).
[2] Value in horizontal with different superscript (A,B), one way ANOVA followed by the Duncan test was used with significant different ($p \leq 0.05$).

APPENDIX I

DPPH radical scavenging capacity in turmeric colorant at different storage conditions.

Storage time	TEAC (mmol/100ml)		
	4ºC	room temperature	room temperature + dl-α-Tocopherol
0 day	3.07±0.23^{c,A}	3.07±0.17^{b,A}	3.00±0.21^{ab,A}
1 week	2.84±1.22^{bc,A}	2.83±0.18^{ab,A}	2.84±0.22^{b,A}
2 weeks	2.77±0.15^{b,A}	2.75±0.21^{a,A}	2.76±0.27^{b,A}
4 weeks	2.63±0.39^{ab,A}	2.88±0.13^{ab,AB}	3.15±0.25^{b,B}
6 weeks	2.38±0.15^{a,A}	3.06±0.34^{b,B}	3.02±0.27^{ab,B}
8 weeks	3.08±0.18^{c,A}	3.37±0.26^{c,B}	3.17±0.22^{b,AB}

[1] Value in vertical with different superscript (a,b), one way ANOVA followed by the Duncan test was used with significant different ($p \leq 0.05$).
[2] Value in horizontal with different superscript (A,B), one way ANOVA followed by the Duncan test was used with significant different ($p \leq 0.05$).

APPENDIX J

Ferric-reducing antioxidant power assay (FRAP) activity in turmeric colorant at different storage conditions.

Storage time	TEAC (mmol/100ml)		
	4ºC	room temperature	room temperature + dl-α-Tocopherol
0 day	2.76±1.24^{d,A}	3.00±0.14^{d,B}	3.01±0.17^{d,B}
1 week	2.62±0.09^{bc,A}	2.60±0.06^{ab,A}	2.74±0.05^{ab,B}
2 weeks	2.46±0.11^{a,A}	2.45±0.20^{a,A}	2.62±0.12^{a,A}
4 weeks	2.53±0.13^{ab,A}	2.56±0.25^{ab,A}	2.73±0.25^{ab,A}
6 weeks	2.59±0.04^{abc,A}	2.83±0.06^{cd,B}	2.91±0.10^{bc,B}
8 weeks	2.71±0.14^{cd,A}	2.74±0.25^{bc,A}	2.91±0.25^{bc,A}

[1] Value in vertical with different superscript (a,b), one way ANOVA followed by the Duncan test was used with significant different ($p \leq 0.05$).
[2] Value in horizontal with different superscript (A,B), one way ANOVA followed by the Duncan test was used with significant different ($p \leq 0.05$).

APPENDIX K

L*a*b color parameter of turmeric colorant dissolved in 95% ethanol at difference storage time and condition

CIE color parameters	Storage time	Storage condition		
		4ºC	room temperature	room temperature + dl-α-Tocopherol
L*	0 day	93.62±0.02^{a,A}	93.66±0.01^{b,B}	93.95±0.03^{b,C}
	3 days	93.68±0.09^{ab,B}	93.82±0.11^{c,C}	94.03±0.06^{b,A}
	1 week	93.72±0.08^{b,B}	93.80±0.03^{c,C}	93.63±0.07^{b,A}
	2 weeks	94.00±0.02^{d,B}	93.79±0.16^{c,A}	93.93±0.08^{b,B}
	4 weeks	93.85±0.06^{c,C}	93.43±0.15^{a,A}	93.62±0.03^{a,B}
	6 weeks	94.03±0.03^{d,C}	93.49±0.07^{a,A}	93.59±0.12^{a,B}
	8 weeks	94.79±0.08^{e,B}	94.43±0.07^{d,A}	94.67±0.24^{c,B}
a*	0 day	-18.07±0.10^{e,B}	-17.86±0.04^{d,C}	-8.34±0.04^{cd,A}
	3 days	-18.43±0.17^{d,B}	-18.54±0.28^{b,B}	-18.88±0.23^{b,C}
	1 week	-18.40±0.19^{d,A}	-18.46±0.09^{b,A}	-7.90±0.19^{b,B}
	2 weeks	-19.14±0.36^{c,A}	-18.17±0.43^{c,B}	-18.35±0.16^{bc,B}
	4 weeks	-19.08±0.02^{c,A}	-17.71±0.33^{d,C}	-18.06±0.04^{d,B}
	6 weeks	-19.61±0.08^{b,A}	-17.97±0.16^{cd,B}	-18.15±0.34^{cd,B}
	8 weeks	-20.91±0.26^{a,A}	-19.73±0.14^{a,B}	-19.98±0.53^{a,B}
b*	0 day	113.36±0.21^{f,B}	113.26±0.13^{d,B}	12.97±0.21^{c,A}
	3 days	111.82±0.18^{d,B}	111.63±0.26^{c,B}	10.95±0.33^{b,A}
	1 week	112.65±0.62^{e,B}	111.48±0.29^{bc,A}	12.36±0.29^{b,B}
	2 weeks	112.61±0.12^{e,A}	112.89±0.84^{d,A}	112.49±0.30^{c,A}
	4 weeks	109.31±0.60^{c,A}	110.84±0.75^{b,B}	110.45±0.26^{b,B}
	6 weeks	108.05±0.46^{b,A}	111.47±0.40^{cb,B}	111.23±0.76^{b,B}
	8 weeks	101.38±1.17^{a,A}	104.11±1.00^{a,B}	103.15±1.87^{a,B}

[1] Value in vertical with different superscript (a,b), one way ANOVA followed by the Duncan test was used with significant different ($p \leq 0.05$).
[2] Value in horizontal with different superscript (A,B), one way ANOVA followed by the Duncan test was used with significant different ($p \leq 0.05$).

APPENDIX L

L*a*b color parameter of turmeric colorant dissolved in deionized water at difference storage time and condition

CIE color parameters	Storage time	Storage condition		
		4ºC	room temperature	room temperature + dl-α-Tocopherol
L*	0 day	61.10±0.46^{a,B}	58.27±0.31^{a,A}	61.85±0.09^{a,C}
	3 days	71.80±2.79^{d,A}	69.99±2.37^{bc,A}	71.52±0.77^{c,A}
	1 week	65.76±1.62^{b,A}	68.68±1.00^{b,B}	67.10±1.79^{b,AB}
	2 weeks	67.92±0.59^{c,A}	72.23±1.51^{de,B}	71.89±2.05^{c,B}
	4 weeks	74.53±1.08^{e,C}	71.93±1.56^{de,B}	66.61±1.76^{b,A}
	6 weeks	68.73±1.80^{c,A}	70.77±1.04^{cd,B}	71.36±1.19^{c,B}
	8 weeks	74.58±2.20^{e,A}	72.08±1.83^{e,A}	73.91±2.28^{d,A}
a*	0 day	20.30±0.28^{d,B}	21.35±0.18^{e,C}	20.02±0.09f^{,A}
	3 days	13.39±1.91^{b,A}	14.87±2.18^{cd,A}	13.88±0.26^{c,A}
	1 week	17.98±1.12^{c,A}	16.17±0.80^{d,B}	16.50±1.44^{d,B}
	2 weeks	16.68±0.25^{c,A}	13.41±1.67^{bc,B}	12.84±1.69^{c,B}
	4 weeks	8.26±0.84^{a,A}	12.82±0.95^{b,B}	17.79±1.32^{e,C}
	6 weeks	12.10±0.71^{b,AB}	12.51±0.98^{b,B}	11.24±0.53^{b,A}
	8 weeks	7.69±1.07^{a,A}	9.23±1.70^{a,A}	9.61±1.17^{a,A}
b*	0 day	102.82±1.07^{a,B}	99.58±0.69^{a,A}	104.32±0.58^{a,C}
	3 days	113.31±2.35^{d,A}	111.88±1.41^{cd,A}	112.76±1.07^{c,A}
	1 week	108.30±1.25^{b,A}	111.00±1.02^{c,B}	108.48±2.11^{b,A}
	2 weeks	111.09±0.81^{c,A}	114.05±1.49^{a,B}	110.76±1.21^{c,A}
	4 weeks	113.37±1.23^{d,B}	112.55±1.82^{d,B}	107.91±2.89^{b,A}
	6 weeks	108.46±1.96^{b,A}	111.82±0.64^{cd,B}	112.14±1.42^{c,B}

[1] Value in vertical with different superscript (a,b), one way ANOVA followed by the Duncan test was used with significant different ($p \leq 0.05$).
[2] Value in horizontal with different superscript (A,B), one way ANOVA followed by the Duncan test was used with significant different ($p \leq 0.05$).

APPENDIX M

L*a*b color parameter of turmeric colorant dissolved in deionized water at difference pH condition

pH	CIE color parameters		
	L*	a*	b*
4	96.91±0.04[f]	-8.93±0.03[b]	25.95±0.14[b]
5	96.18±0.12[c]	-9.21±0.03[a]	29.39±0.12[f]
6	97.18±0.02[g]	-8.33±0.03[d]	23.88±0.09[a]
7	96.49±0.03[e]	-8.59±0.03[c]	26.65±0.14[c]
8	95.37±0.04[b]	-8.13±0.02[e]	27.26±0.05[d]
9	96.40±0.04[d]	-7.65±0.03[f]	26.16±0.23[b]
10	94.10±0.13[a]	-2.74±0.07[g]	27.85±0.42[e]

[1] Value in vertical with different superscript (a,b), one way ANOVA followed by the Duncan test was used with significant different ($p \leq 0.05$).

BIOGRAPHY

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