EXTRACTION OF A NATURAL COLORANT FROM DRAGON FRUIT PEEL AND DETERMINATION OF ITS PROPERTIES

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ABSTRACT

At present there is a growing trend of using natural colorants in foods, because consumers are concerned about the food quality and health. Dragon fruit peel was investigated due to its high betalain content. The study aimed to prepare a natural colorant concentrate by extraction from dragon fruit peel, determine its properties with an emphasis on the antioxidant activity, and apply the extracted colorant in food products. Dragon fruit peel was extracted with 95% ethanol and evaporated under vacuum. The dragon fruit (DF) colorant was dark red in color. Its properties were determined by color parameters, betacyanin content, and antioxidant activity. The colorant stability was studied during storage at 4 °C for 2 months. At day 0, betcyanin content was 1.25 mg/ml. The antioxidant activities determined by DPPH and ORAC assay were 19.37±0.08 and 222.18±25.85 μmol TE/ml, respectively. These values declined with storage time due to degradation of betacyanin. The effect of pH on the stability of DF colorant was studied at pH 3, 5, and 7. It exhibited the highest stability at pH 5. Application of DF colorant in pasteurized milk and jelly were tested and compared with a synthetic food colorant by sensory evaluation. The results of sensory evaluation showed that the foods added with DF colorant were accepted by the panelists. Hence, a natural colorant with antioxidant activity could be prepared from dragon fruit peel and applied in food products as an alternative to a synthetic colorant.

KEY WORDS: DRAGON FRUIT PEEL/ BETALAIN/ BETACYANIN/ FOOD COLORANT/ ANTIOXIDANT

111 pages

การสกัดและการศึกษาสมบัติของสีธรรมชาติจากเปลือกแก้วมังกร
EXTRACTION OF A NATURAL COLORANT FROM DRAGON FRUIT PEEL AND DETERMINATION OF ITS PROPERTIES

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บทคัดย่อ

ในปัจจุบันมีแนวโน้มในการใช้สีผสมอาหารจากธรรมชาติมากขึ้นเนื่องจากผู้บริโภคให้ความตระหนักในเรื่องคุณภาพอาหารและสุขภาพมากขึ้น ทั้งนี้ได้มีการพบว่าในเปลือกแก้วมังกรมีปริมาณบีต้าเลนสูง งานวิจัยนี้จึงมี จุดประสงค์เพื่อผลิตสีผสมอาหารซึ่งสกัดจากเปลือกแก้วมังกร วิเคราะห์คุณสมบัติโดยเน้นทางด้านฤทธิ์การต้านอนุมูลอิสระและทดสอบในผลิตภัณฑ์อาหาร เปลือกแก้วมังกรถูกสกัดด้วย 95% เอทานอล สีเข้มข้นจากเปลือกแก้วมังกรที่สกัดได้เป็นสีแดงเข้ม และได้ถูกทำการวิเคราะห์ค่าสี ปริมาณเบต้าไซยานิน และฤทธิ์การต้านอนุมูลอิสระ สีจากเปลือกแก้วมังกรได้ถูกศึกษาความคงตัวโดยเก็บรักษาที่อุณหภูมิ 4 องศาเซลเซียส เป็นเวลา 2 เดือน ในวันแรกของการเก็บรักษา สีจากเปลือกแก้วมังกรมีปริมาณเบต้าไซยานิน 1.25 มิลลิกรัมต่อมิลลิลิตร มีฤทธิ์การต้านอนุมูลอิสระ 19.37±0.08 และ 222.18±25.85 ไมโครโมลโทรลอกซ์ต่อมิลลิลิตร ซึ่งทำการวิเคราะห์โดยวิธี DPPH และ ORAC ตามลำดับ ปริมาณเหล่านี้ได้ลดลงตามระยะเวลาการเก็บรักษาเนื่องจากการเสื่อมสลายของเบต้าไซยานิน นอกจากนั้นได้ทำการศึกษาผลของ pH ต่อความคงตัวของสีที่ pH แตกต่างกันคือที่ pH 3, 5 และ 7 จากการทดลองพบว่าสีจากเปลือกแก้วมังกรที่ pH 5 มีความคงตัวสูงที่สุด ในส่วนของการประยุกต์ใช้ในอาหาร ได้ทำการทดสอบใช้สีจากเปลือกแก้วมังกรในนมพาสเจอร์ไรส์และเยลลี่เปรียบเทียบกับการใช้สีสังเคราะห์ โดยการประเมินทางประสาทสัมผัสพบว่าผู้บริโภคให้การยอมรับในการใช้สีจากเปลือกแก้วมังกรในทั้งสองผลิตภัณฑ์ ดั้งนั้นสีจากธรรมชาติสกัดจากเปลือกแก้วมังกรซึ่งมีฤทธิ์ในการต้านอนุมูลอิสระสามารถประยุกต์ใช้ได้ในผลิตภัณฑ์อาหารหลายชนิด จึงเป็นทางเลือกหนึ่งในการเลือกใช้เพื่อทดแทนการใช้สีผสมอาหารสังเคราะห์

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LIST OF ABBREVIATIONS

DPPH	2,2-diphenyl-1-picrylhydrazyl
ORAC	oxygen radical absorbance capacity
AAPH	2,2’-azobis (2-amidinopropane) dihydrochloride
AUC	net area under curve
HPLC	High-performance liquid chromatography
°C	degree Celcius
g	gram
mg	milligram
l	liter
ml	milliliter
μl	microliter
μmol TE	micromole of trolox equivalent
cm	centimeter
nm	nanometer
w/v	weight by volume
min	minute
s	second
bw	body weight

CHAPTER I

INTRODUCTION

The color of foods is an important quality indicator of the consumer acceptability in food products. The market demand for attractive products has led to the development and use of food colorants by the food manufacturing industries. Food colorants are categorized into 4 groups (1). They are 1) organic synthetic colorant such as Ponceau 4 R (red), Tartrazine (yellow), Fast green FCF (green), and Brilliant blue FCF (blue), 2) inorganic colorant such as vegetable charcoal and titanium dioxide, 3) natural colorant from plant or animal extract such as the colorant from carotenoids, betalain, chlorophyll, and 4) food color additives other than the colors in category 1, 2, and 3. Nowadays, more and more interest has been directed to the color in category 3 i.e. natural colorants which are considered as a safe and preferred choice compared to synthetic colorants by the consumers.

A growing trend of using natural colorants came along because people are increasingly concerned about the food quality, diet and health. Moreover, there have been many studies reporting that synthetic food colorants could exhibit carcinogenicity, genotoxicity and neurotoxicity. According to long-term studies in animals, it was found that several symptoms occurred in the test animals fed with foods containing synthetic colorants such as adrenal atrophy, chronic follicular cystitis, diarrhea, and pyloric gastritis. Most natural colorants have no negative effects on animals (2). Hence, application of natural colorants in food products could be beneficial as well as provide another alternative for the consumers.

The majority of natural colorant is obtained from plant extract. Plants are good sources of pigments or bioactive compounds which can be utilized as natural colorant. Examples of these pigments include 1) chlorophylls (green pigment) from all plants, algae, or ferns, 2) carotenoids (red-yellow pigment) from carrot, tomato, or strawberry, 3) anthocyanin (red-purple pigment) from mulberry, grape, or red onion, 4) betalain (yellow-red pigment) from cactus pear, dragon fruit, red beet, and many more. Currently some of these plants are used to produce natural food colorants in the industry (2).

Dragon fruit (*Hylocereus spp.)* is abundantly produced in Thailand. This fruit is rich in vitamin C and phosphorus. It has high antioxidant activity especially the red flesh species, *Hylocereus polyhizus*. According to traditional belief, it possesses medicinal properties such as deterring colon cancer and diabetes, reducing cholesterol and high blood pressure and controlling blood sugar level (3). Moreover, betalain pigment, insoluble dietary fiber, and soluble dietary fiber particularly pectin were found in very high level in its peel (4). Hence, dragon fruit peel could be utilized as a good source of natural colorant due to its high betalain content.

Betalain is a natural water-soluble pigment. It is approved for food use in several countries including the European Union (EU) and the United States of America (USA), where betalain is labeled as E-162 and 73.40, respectively (5). Betalain can be divided into two subclasses based on the structural characteristics. They are betacyanin (red-purple) and betaxanthin (yellow). These natural colors can be found in fruits or vegetables such as red/yellow beet, cactus pear, amaranth and dragon fruit (6). Betalain is an unstable molecule and it is easily degraded during food processing. The important factors affecting the stability of betalain are temperature, pH, oxygen and light (7). Consequently, extraction and storage condition or food processing can result in a profound effect on the stability of colors and their antioxidant activity. Therefore, it was necessary to investigate the factors affecting the stability and quality of the colorant. This study aimed to prepare a natural colorant concentrate by extraction from the peel of red flesh dragon fruit and determine its properties with an emphasis on its antioxidant activity. Betacyanin was expected as the major pigment in the dragon fruit peel colorant. Moreover, the study also determined the stability of dragon fruit peel colorant during storage as well as its application in certain food products.

CHAPTER II

OBJECTIVES

2.1 General objective

To prepare a natural colorant concentrate by extraction from the peel of red flesh dragon fruit, determine its properties, and investigate its potential application in food products

2.2 Special objectives

1. To study the suitable condition for extraction of betacyanin from dragon fruit peel
2. To study the properties of dragon fruit peel colorant under various conditions
3. To study the stability of dragon fruit peel colorant during storage
4. To determine the betacyanin content of dragon fruit peel colorant by spectrophotometry method
5. To determine the antioxidant activity of dragon fruit peel colorant by DPPH and ORAC assay
6. To apply the dragon fruit peel colorant in food products and evaluate the consumer acceptability

2.3 Expected outcomes

1. Dragon fruit peel could be used as a source of natural colorant.
2. The properties of dragon fruit peel colorant (betacyanin content, antioxidant activity, and color parameters) were studied.
3. Dragon fruit peel colorant could be applied in food products.

CHAPTER III
LITERATURE REVIEW

3.1 Food colorants

Food colorants have been used since the early civilization and the use became massive in 19th century. Nowadays, food colorants are used in commercial food production and also in household cooking. The first fascination toward many food products undeniably comes from their color. The color of a food product is an important indicator for the consumer acceptance in term of preference or as an implication of the quality of the product. The consumers recognize color of food, for example, meat should be red, margarine must be yellow, or blueberry must be purple. The color of food can also be associated with food safety regarding spoilage, processing, and transportation (2).

3.1.1 Definition

A food colorant is any dye, pigment or substance synthesized, extracted, isolated or otherwise derived from plants, animals, or other sources which added or applied to food or beverage to create or modify its color. It is added in food products for many reasons such as to offset the color loss caused by processing (heating, exposure to light and/or oxygen, etc.), to correct natural variations in color, to enhance colors that occur naturally, or for decorative purposes (8, 9).

3.1.2 Classification of food colorant

Food colorants can be categorized in many ways by different basis. Most common classifications are based on their origin and legislation at present. Based on origin, they are classified into 3 types as follows (2):

a. Natural colorant: The organic compounds obtained from live organisms; carotenoids, anthocyanins, and curcumin.

b. Synthetic colorant: The organic compounds obtained by chemical synthesis; Allura red, Sunset yellow, and Erythrosine.

c. Inorganic colorant: The colorant obtained by synthesis of natural substance; titanium dioxide.

Based on the United States legislation, they are categorized into 2 groups which are

a. Certifiable colorant: Anthropogenic synthetics colorant

b. Exempt from certification colorant: Colorant obtained from natural origin or synthetic counterparts which do not require certification.

On the other hand, according to the Notification of the Ministry of Public Health (Thailand) Number 21, B.E. 2522, food colorants are classified into 4 groups by their origin as follows (1):

a. Organic synthetic colorants: Ponceau 4 R (red), Tartrazine (yellow), Fast green FCF (green), and Brilliant blue FCF (blue)

b. Inorganic colorants: Titanium dioxide and vegetable charcoal

c. Natural colorants extracted from plant or animal: Carotenoids, betalain, chlorophyll which are not harmful for consumption

d. Food color additives consisting of the organic synthetic, inorganic, or natural colorant from plant or animal extracted colorant in category a, b, and c.

3.1.3 International legislation of food colorant

3.1.3.1 United States legislations (US legislations)

In the United States, food colorants have been used for a long time without any regulations. The US Congress had approved the special legislation for food additives which was commissioned by US Department of Agriculture (USDA) in 1904. The legislation for food additives have been approved continuously since that time (2). The Federal Food, Drug, and Cosmetic Act (FD&C Act) is a part of the US legislation on food additives. Food colorants have to be permitted by the Food & Drug Administration (FDA) otherwise the FD&C Act infers foods containing those colorants as debased foods. The usage of colorants is controlled by the Code of

Federal Regulations (CFR). The FDA established the regulations for food colorants in Title 21 of the CFR in part 70, 71, 73, 74, and 80-82. They cover different subjects concerning food colorants i.e. safety evaluation of colorant, labeling requirements, color additive certification, and so on.

According to the regulation, color additives have been categorized into 2 types based on the legislation as mentioned in section 3.1.2. The certifiable colorants are given the different name codes instead of the commercial names. There are 3 categories as follows:

FD&C colorants – Naming for certifiable colorants which can use in foods, drugs, and cosmetics.

D&C colorants – Naming for certifiable colorants are allowed for use in drugs and cosmetics.

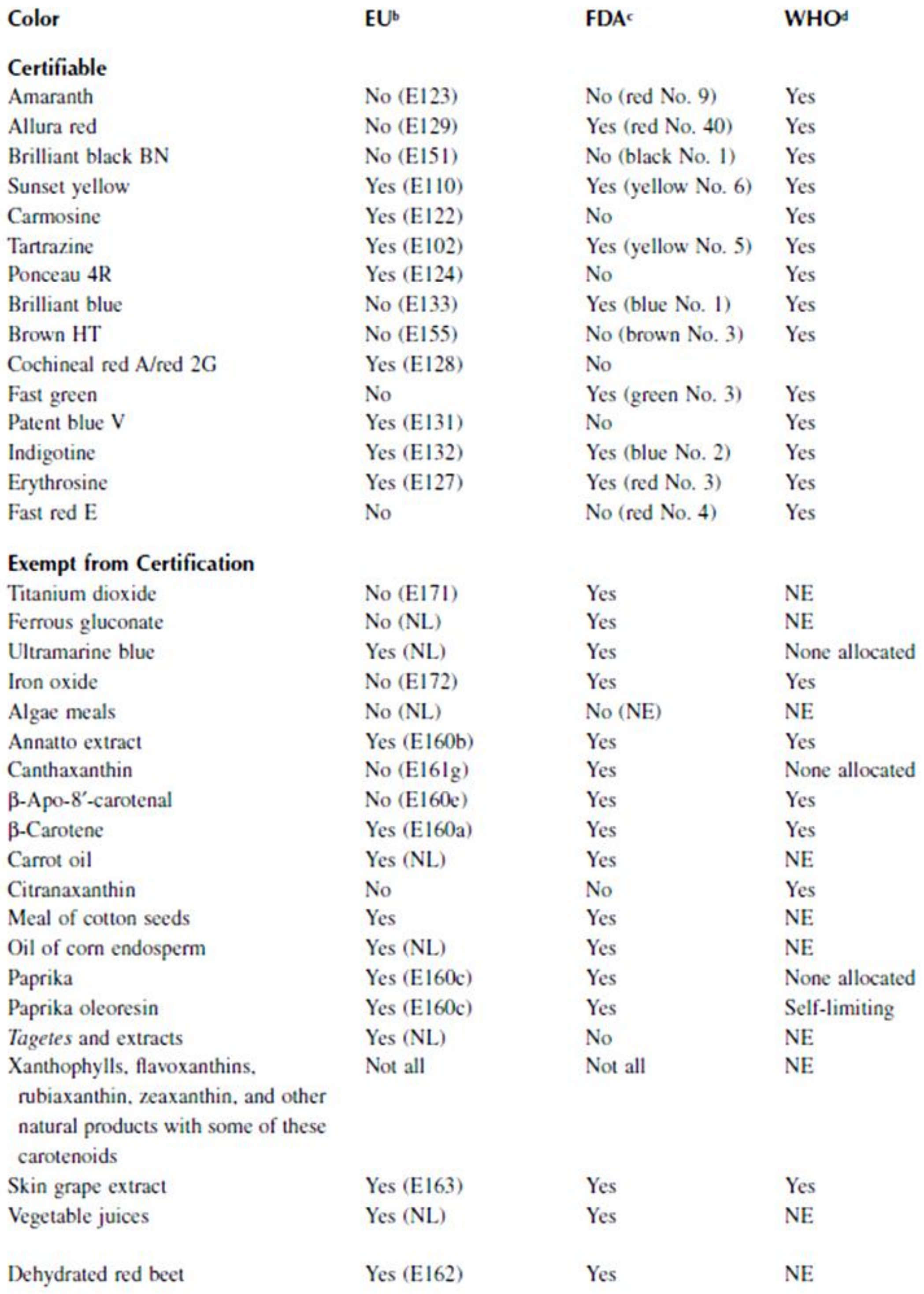
Ext. D&C colorants – Naming for the colorants approved for use in drugs and cosmetics which are externally applied.

For example, FD&C Red No.3 – A red colorant intended for use in foods, drugs, and cosmetics. “FD&C” exhibits the intended use, “Red” relates to its hue, and “No.3” distinguishes it from other red colorants. Only 7 color additives are approved by the US FDA and are listed in **Table 3.1**. On the other hand, 26 color additives of exempt from certification colorants are approved and named based on their common name, chemical structure, or their origin such as annatto, betalain, paprika, etc. (9, 10).

3.1.3.2 European Union legislations (EU legislations)

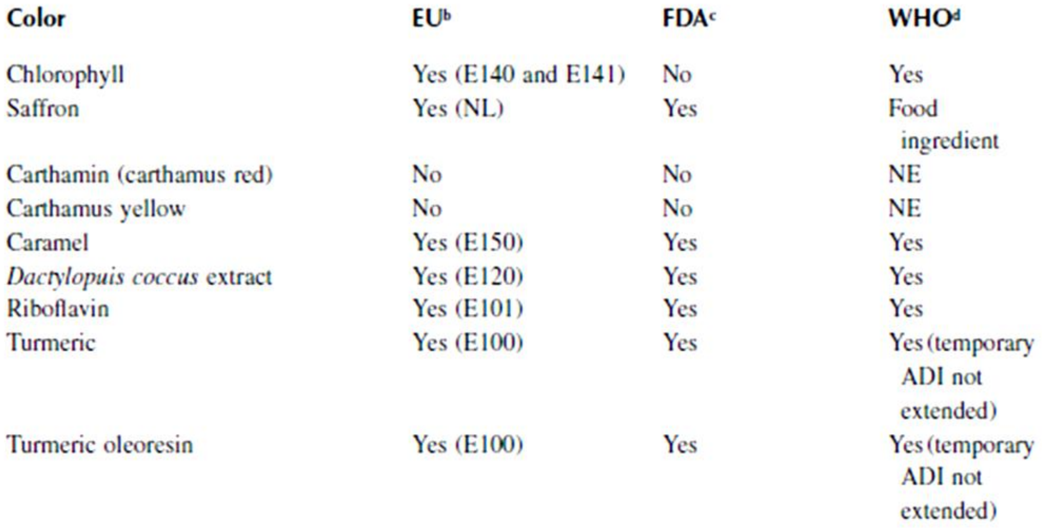
In the EU legislations, all food additives are identified by E number (E=EEC number of food additives). Color additive is a kind of food additives thus it also carries the E number. There are 43 colorants, 17 synthetic colorants and 26 natural or nature identical colorants, approved for food use by the EU legislations e.g. E140 chlorophyll, E150 caramel, or E124 Ponceau 4R. The EU directives are based on the recommendations of the Codex Alimentarius, the Scientific Committee for Food (SCF), and the Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA). The legislation is harmonized across the EU among member states. However, each member state can also have its own domestic legislation depending on the various directives.

Table 3.1 Approved Colors for the Food and Feed Industries by the European Union (EU), by the U.S. Food and Drug Administration (FDA), and by the World Health Organization (WHO) (2)



Color	EU[b]	FDA[c]	WHO[d]
Certifiable			
Amaranth	No (E123)	No (red No. 9)	Yes
Allura red	No (E129)	Yes (red No. 40)	Yes
Brilliant black BN	No (E151)	No (black No. 1)	Yes
Sunset yellow	Yes (E110)	Yes (yellow No. 6)	Yes
Carmosine	Yes (E122)	No	Yes
Tartrazine	Yes (E102)	Yes (yellow No. 5)	Yes
Ponceau 4R	Yes (E124)	No	Yes
Brilliant blue	No (E133)	Yes (blue No. 1)	Yes
Brown HT	No (E155)	No (brown No. 3)	Yes
Cochineal red A/red 2G	Yes (E128)	No	
Fast green	No	Yes (green No. 3)	Yes
Patent blue V	Yes (E131)	No	Yes
Indigotine	Yes (E132)	Yes (blue No. 2)	Yes
Erythrosine	Yes (E127)	Yes (red No. 3)	Yes
Fast red E	No	No (red No. 4)	Yes
Exempt from Certification			
Titanium dioxide	No (E171)	Yes	NE
Ferrous gluconate	No (NL)	Yes	NE
Ultramarine blue	Yes (NL)	Yes	None allocated
Iron oxide	No (E172)	Yes	Yes
Algae meals	No (NL)	No (NE)	NE
Annatto extract	Yes (E160b)	Yes	Yes
Canthaxanthin	No (E161g)	Yes	None allocated
β-Apo-8′-carotenal	No (E160e)	Yes	Yes
β-Carotene	Yes (E160a)	Yes	Yes
Carrot oil	Yes (NL)	Yes	NE
Citranaxanthin	No	No	Yes
Meal of cotton seeds	Yes	Yes	NE
Oil of corn endosperm	Yes (NL)	Yes	NE
Paprika	Yes (E160c)	Yes	None allocated
Paprika oleoresin	Yes (E160c)	Yes	Self-limiting
Tagetes and extracts	Yes (NL)	No	NE
Xanthophylls, flavoxanthins, rubiaxanthin, zeaxanthin, and other natural products with some of these carotenoids	Not all	Not all	NE
Skin grape extract	Yes (E163)	Yes	Yes
Vegetable juices	Yes (NL)	Yes	NE
Dehydrated red beet	Yes (E162)	Yes	NE

Table 3.1 (cont.) Approved Colors for the Food and Feed Industries by the European Union (EU), by the U.S. Food and Drug Administration (FDA), and by the World Health Organization (WHO)



Color	**EU**[b]	**FDA**[c]	**WHO**[d]
Chlorophyll	Yes (E140 and E141)	No	Yes
Saffron	Yes (NL)	Yes	Food ingredient
Carthamin (carthamus red)	No	No	NE
Carthamus yellow	No	No	NE
Caramel	Yes (E150)	Yes	Yes
Dactylopuis coccus extract	Yes (E120)	Yes	Yes
Riboflavin	Yes (E101)	Yes	Yes
Turmeric	Yes (E100)	Yes	Yes (temporary ADI not extended)
Turmeric oleoresin	Yes (E100)	Yes	Yes (temporary ADI not extended)

[a] This table lists the most common approved colorants.

[b] To simplify the terminology, EU assigns a code to identify those additives that were evaluated. The letter “E” precedes code number. NL = EU has not assigned a code number and current legislation does not cover the corresponding pigment.

[c] In parenthesis are shown other common names.

[d] ADI = acceptable daily intake; NE = pigment with a “not specified” ADI. It is used in accordance with Good Manufacturing Practices (GMP). However, NE does not mean that unlimited intake is acceptable. It means that at the levels used to achieve the desired effect and from acceptable background in food it does not, in the opinion of the JECFA, represent a hazard for health. Thus, if a substance will be used in larger amounts and/or in a wider range of foods than envisaged by JECFA, it may be necessary to obtain approval of the committee.

None allocated = JECFA has been unable to allocate an ADI but nevertheless found a specific use of a substance acceptable. Thus, no allocated additives are authorized in accordance with the conditions specified. If conditions are modified, additives must be reevaluated and approved by the JECFA.

There are many directives for the community legislation of food additives; three of those directives involve color additives (2, 10).

Council Directive 89/107/EEC, amended by Directive 94/34/EC, provides the framework for the authorization of food additives. It consists of a definition of food additive, exclusion from the scope of the definition, list of food additive categories, and the general criteria for using of food additives.

European Parliament and Council Directive 94/36/EC: This directive mentions the detail of rules on the colorants. It includes the list of permitted colors, foodstuffs which only a limited list of colorants can be used, basic food stuffs that are not allowed to add any colorants and colorants that have restricted application.

Commission Directive 95/45/EC, amended by Directive 99/75/EC for colors, details that authorized food colorants must fulfill purity criteria (10).

In addition, there are some directives which mention on the chemical and environmental issues relevant to dyes such as Directive for the Classification, Packaging, and Labeling of Dangerous Substances (67/548/EEC) or Directive on the Control of Major Accident Hazards Involving Dangerous Substances (96/82/EC) (11).

Furthermore, each country has different standards for using color additives in different food products which are authorized by the legislation of that country. Many researchers reported that color additives (Sunset yellow E110, Carmoisine E122, Tartrazine E102, Ponceau 4R E124, Allura red E129, and Quinoline yellow E104) caused the attention deficit hyperactivity disorder (ADHD) in children. In 2009, European Food Safety Authority (EFSA) collected available information and supported changing the control of using color additives. This led to the launching of the regulation (EC) No 1333/2008. This regulation mentions that all foods added with those 6 colorants must carry a warning label “May have an adverse effect on activity and attention in children” (12). Moreover, the Korea Food & Drug Administration (KFDA) under the Ministry of Health and Welfare (MHW) prohibits the use of 14 tar food colorants especially in children’s preferred food such as candy, ice-cream, milk, carbonated beverage, etc. (13).

3.1.4 Commercial synthetic colorants with red-purple color

Ponceau 4R: Ponceau 4r is also known as CI Food Red 7, Cochineal Red A, New Coccine, Brilliant Scarlet, CI (1975) No. 16255, and E No. 124 (CI = Color Index). Its CAS number, a group of number set by Chemical Abstract Service, is 2611-82-7. Its chemical name is trisodium 2-hydroxy-1-(4-sulfonato-1-naphthylazo)-6, 8-naphthalenedisulfonate ($C_{20}H_{11}N_2Na_3O_{10}S_3$). The formula weight is 604.48. It is a water soluble colorant and is sparingly soluble in ethanol. The general appearance is reddish powder or granules. For UV-visible absorption, the maximum absorption wavelength is between 505 to 510 nm. Ponceau 4R has been evaluated by JECFA and the EU Scientific Committee for Food (SCF). They gave an acceptable daily intake (ADI) of 0-4 mg/kg body weight (bw)/day. Furthermore, in the US and many countries, this colorant is a suspected carcinogen and is prohibited for use in food products (14-16).

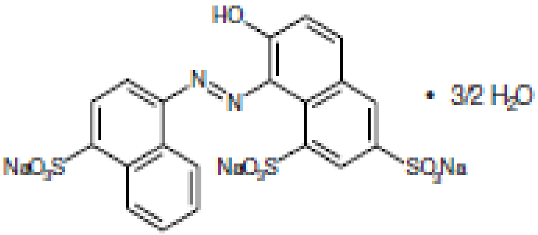


Figure 3.1 Chemical structure of Ponceau 4r (2)

Allura red: The synonyms of allura red are CI Food Red 17, FD&C Red No. 40, CI (1975) No. 16035, and E No. 129. Its CAS number is 25956-17-6. The chemical name is disodium 6-hydroxy-5-(2-methoxy-5-methyl-4-sulfonato-phenylazo)-2-naphthalene-sulfonate ($C_{18}H_{14}N_2Na_2O_8S_2$) and the formula weight is 496.43. Allura red appears in dark red powder or granules which are water soluble and ethanol insoluble. The maximum absorption wavelength is 504 nm. JECFA and SCF established an ADI of 0-7 mg/kg bw/day. A previous report mentioned that allura red caused DNA damage, in vivo, in the glandular stomach, lungs, and colon of mice (17, 18). The chemical structure of allura red is illustrated in **Figure 3.2**.

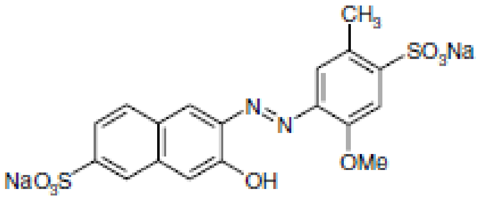


Figure 3.2 Chemical structure of allura red (2)

Erythrosine: The synonyms of erythrosine are CI Food Red 14, FD&C Red No. 3, CI (1975) No. 45430, and E No. 127. The CAS number is 16423-68-0. Erythrosine is a xanthenes dye represents bluish pink color. Its chemical name is disodium salt of 9-(*o*-carboxyphenyl)-6-hydroxy-2,4,5,7-tetraiodo-3-isoxanthone monohydrate ($C_{20}H_6I_4Na_2O_5 \cdot H_2O$). The formula weight is 897.88. Erythrosine is a red powder or granules which can be dissolved in both water and ethanol. The maximum absorption wavelength is 526 nm by UV-visible spectrophotometry. The ADI of erythrosine is 0-0.1 mg/kg bw/day. Erythrosine is not only used in food products, but also can be used in inks and as a dental plaque disclosing agent (15, 19, 20). The chemical structure of erythrosine appears in **Figure 3.3**.

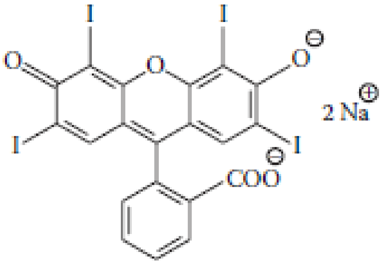


Figure 3.3 Chemical structure of erythrosine (15)

3.1.5 Natural colorant

A natural colorant is a colorant or dye derived from living organisms such as plants, fungi, and animals or from minerals. Most natural colorants can be obtained from parts of plant, for instance, root, bark, leaves, fruits, and many more. Natural colorants, which are well known in general plants and applied as food, drug and cosmetic additives, include chlorophylls, carotenoids, anthocyanins, etc. (2).

Chlorophylls: Chlorophylls are the green pigment from photosynthetic organisms. They provide the color ranging from yellow-green to blue-green. Chlorophylls are sensitive to heat and storage time. The color degrades immediately after harvesting with the rate depending on the process condition. They are the most abundant pigments in nature and can be found in all plants, fern, algae, and mosses. Codex legislation approved chlorophylls in various food applications, for example, dairy products, cereal and starch-based desserts, wines, smoked marine products, and many more (2, 21, 22). The structure of chlorophyll is illustrated in **Figure 3.4**.

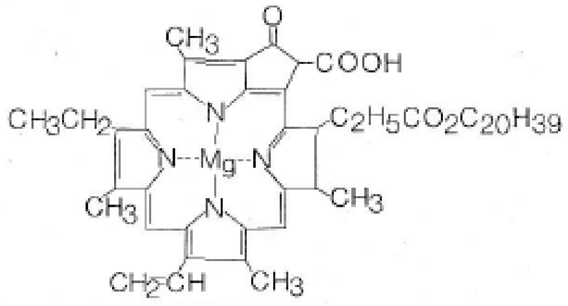


Figure 3.4 Structure of chlorophyll (23)

Carotenoids: Carotenoids are compounds constituted by 8 isoprenoid units. They contain a group of liposoluble pigments that represent red, orange, and yellow color. They are classified based on the chemical structure into 2 groups that are carotenes and xanthophylls. Carotenoids are widespread in nature especially in plant sources such as carrot, papaya, tomato, kale, broccoli, etc. Moreover, they have been used as food colorants for a long time. Some of the most common carotenoids sources are saffron, annatto, pepper, paprika, and carrot extracts. They are traditionally used around the world. Carotenoids are not only used as a food colorant, but they are also good sources of antioxidants and vitamin A. However, there might be loss of carotenoids during food processing while in some instances, it sometimes increases the bioactive compounds. Some studies reported that carotenoids could inhibit mutation and exhibited a low acute oral toxicity in mice. There is no ADI for consumption of carotenoids because of their benefits (2, 24, 25). The structures of some common carotenoids are shown in **Figure 3.5**.

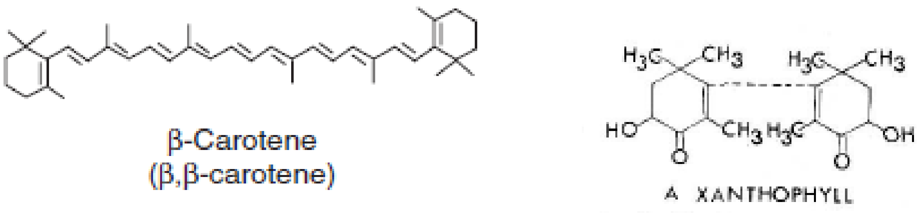


Figure 3.5 Some common carotenoids structures (2,23)

Anthocyanins: Anthocyanins are a group of water-soluble pigments which represent red, purple, and blue color. They belong to the group of flavonoid compounds. The structure of anthocyanins constitutes one or more sugar molecules bonded at different hydroxylated positions of basic structure. Anthocyanins also have important properties such as antioxidant activity or defense mechanisms. They do not show genotocixity. Researchers found that consumption of anthocyanin-rich foods could reduce the risks of cancers. Fruits and vegetables i.e. grape, mulberry, purple corn, or red cabbage are good sources of anthocyanins. In commercial, grape extracts are primarily used as an anthocyanin food colorant in food products such as beverages, confectionery products, and dairy products (2, 24, 26). The structures of some common anthocyanins are shown in **Figure 3.6**.

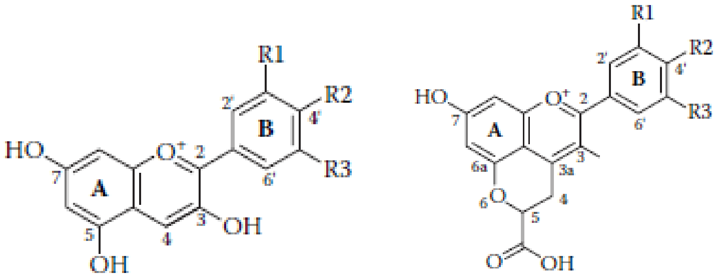


Figure 3.6 Some common anthocyanin structures (27)

Betalains: Betalains are immonium derivatives of betalamic acid. Their colors are in the range of yellow-red and purple. This pigment can be extracted from red beet which is a good source of betalain. Other fruits that contain high amount of betalain include cactus pear fruits (*Opuntia* spp.), Amaranthus which are available in Europe, and also dragon fruit (28-30). The details of betalains are shown in Section 3.3.

3.1.6 Current issues related to colorants

Several studies have reported the possible toxicity of synthetic colorants. It was found that various symptoms occurred in experimental animals fed with foods containing these colorants in long-term studies such as adrenal atrophy, chronic follicular cystitis, diarrhea, and pyloric gastritis. On the other hand, most natural colorants have no negative effects on animals (2). Natural colorants may also be beneficial to health due to the high content of phytochemicals in the fruits or vegetables that were extracted into the colorant along with the pigments. These colorant extracts had been shown to reduce the risk of cancer, cardiovascular diseases, age related eye degradation, and other illnesses. At present, some nutraceutical companies often promote the natural colorants for their products as the functional ingredients (31, 32). Nevertheless, both natural and synthetic color additives should be used appropriately in food products.

3.2 Dragon fruit

In Thailand, dragon fruit is available for more than 10 years. It was brought into the country from Vietnam and the variety was improved for suitability of cultivation under the growing conditions in Thailand. Nowadays dragon fruit can grow well in almost all areas in Thailand, especially in the east of Thailand. It is easy to grow, has fewer pests and provides enormous product yield per plantation. Hence, dragon fruit is popular among the gardeners (33). The data showed that dragon fruit can produce its fruit at the highest amount around 8-12 tons/rai (34). One district in the north of Thailand has cultivated area around 2,000 rai where the gardeners can produce 2,000-3,000 tons of dragon fruit per year (35). Furthermore, a report showed the production of dragon fruit is around 2,000 tons from 7,200 dragon fruit plants (36). These are the examples of cultivated area of Thailand which represented the high volumes of dragon fruit production.

3.2.1 Taxonomy of dragon fruit

Dragon fruit is known as dragon fruit, pitaya, night blooming cactus, or strawberry pear. The common names of dragon fruit are different depending on the languages such as thang loy (Vietnamese), pitaya roja (Spanish), la pitahaya rouge (French), keaw mung korn (Thai), and others as shown in **Table 3.2**. The scientific name of dragon fruit is *Hylocereus* spp. The systematic classification of dragon fruit is presented in **Table 3.3** (3, 37).

More than 25 species of dragon fruit are identified based on different stem and fruit characteristics. The two major species classified by the flesh color are *Hylocereus undatus* (white flesh) and *Hylocereus polyhizus* (red flesh). Furthermore, they are divided into 2 groups based on the stem habit which are vine cacti and columnar cacti (3). The different species of dragon fruit are shown in **Table 3.4** and **Figure 3.7**.

Table 3.2 Common names and synonyms of dragon fruit (3)

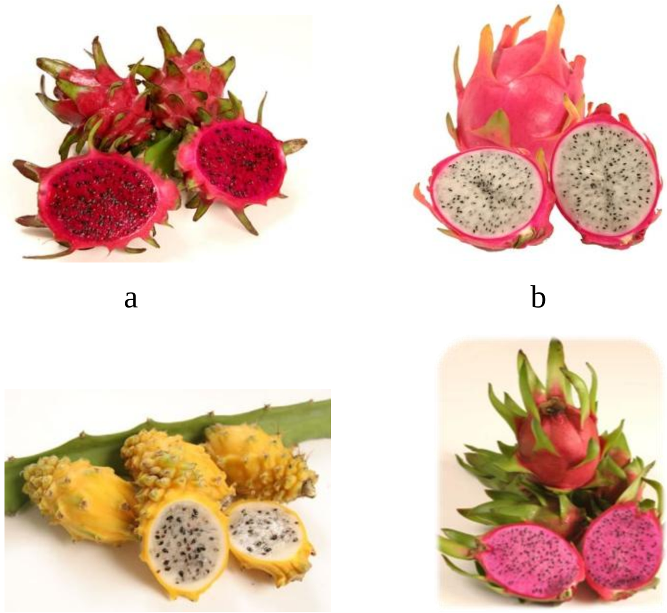
Country/language	**Common name**
Thailand	Keaw mung korn
English	Strawberry pear, Dragon fruit, Red pitaya, Red pitahaya, Night blooming cereus, Belle of the night
Chinese	Zunlongguo
French	Belle de nuit, Cierge-lezard, Pithaya rouge, Poire de chardon
German	Distelbrin, Echte stachelbrin
Indonesian	Buah naga
Spanish	Chaca, Chak-wob, Pitahaya, Pithaya orejona, Nopal, Junco tapatio
Sri Lanka	Dragon fruit
Israel	Pitaya
Hawaii	Paniniokapunahou, Papipi pua, Panani o ka
Vietnam	Dragon fruit, Thanh long
Swedish	Distelbirm, Echtestachelbrin, Rud pitahaya

Table 3.3 The systematic classification of dragon fruit (3)

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta (flowering plants)
Class	Magnoliopsida (dicotyledons)
Subclass	Caryophyllidae
Order	Caryophyllales
Family	Cactaceae (cactus family)
Subfamily	Cactoideae
Genus	*Hylocereus* (Berger) Britt & Rose.

Table 3.4 Classification of different dragon fruit species based on nature of stem habit, flesh, and peel color (3)

Species	**Color**	
	Fruit peel	**Fruit flesh**
Vine cacti		
Hylocereus undatus	red	white
Hylocereus undatus	red	red
Hylocereus triangularis	yellow	white
Hylocereus costaricensis	red	red
Hylocereus polyrhizus	red	red
Hylocereus ocamponis	yellow	red
Selenicereus megalanthus	yellow	white
Columnar cacti		
Cereus triangularis	yellow	white
Acanthocereus jitajaya	yellow	white
Cereus ocamponis	red	red



a b

c d

Figure 3.7 Certain species of dragon fruit; *Hylocereus polyrhizus* (a), *Hylocereus undatus* (b), *Selenicereus megalanthus* (c), *Hylocereus polyrhizus x H. undatus* (d) (38)

3.2.2 Morphological description of plant

Dragon fruit is widely available in Southeast Asia such as Vietnam, Malaysia, and Thailand. It is a tropical cactus fruit originated from the rainforest of central and northern South America. It is now grown commercially in Australia, Israel, Vietnam, Malaysia, and Thailand.

Dragon fruit is a climbing vine cactus species. Its stem is fleshy and fast growing. It may grow up to 6 m or more depending on the growing conditions. Its fruit is medium to large size around 200-500 g per fruit and is an oblong shaped epigenous berry. The peel of dragon fruit is bright red skin or yellow with green scales whereas its flesh is juicy and white or red with tiny black seeds. The unripe fruit is green and contains mucilage. It turns into red color about 25 days after anthesis. Dragon fruit grows well with the optimal conditions i.e. the temperature at 20-30 °C, well-drained soil, and pH 5.5-6.5. It will stop or slow down growing in the warm climate or dry season. The harvesting time varies depending on the area. It is usually from June to December about 40-50 days after flowering. It is harvested when it is mature because it is a non-climacteric fruit (3, 37, 39).

Figure 3.8 Dragon fruit plant (40)

3.2.3 Consumption and nutritional composition of dragon fruit

Dragon fruit can be consumed as ripe fresh fruit. Its taste is mildly sweet and slightly sour. It can be processed into many products such as juice, jam, ice cream, yoghurt, and wine. In Thailand, people consume dragon fruit in fresh fruit form and processed products, for instance dragon fruit flake, candy, jam, juice, and dragon fruit in syrup. These products are generally produced by the small scale industry (41).

Dragon fruit is considered to be a nutritious food because it contains several essential compounds such as niacin, iron, thiamine, and dietary fiber. Furthermore, it has high content of vitamin C, phosphorus, and calcium as shown in **Table 3.5**. The red flesh can be used as a food colorant because it contains high content of betalain (3). The carbohydrates of the flesh consist of glucose, fructose, and oligosaccharides. A previous research also indicated that it has a prebiotic property. It was reported that the oligosaccharides can stimulate the growth of lactobacilli and bifidobacteria (42).

Table 3.5 Chemical composition of dragon fruit in different species (3)

Composition	Amount per 100 g flesh		
	Hylocereus polyrhizus	***Hylocereus undatus***	***Selenicereus magalanthus***
Water (g)	82.5- 83	89.4	85.4
Protein (g)	0.159- 0.229	0.5	0.4
Fat (g)	0.21- 0.61	0.1	0.1
Fiber (g)	0.7- 0.9	0.3	0.5
Carotene (mg)	0.005- 0.012	-	-
Calcium (mg)	6.3- 8.8	6	10
Phosphorus (mg)	30.2- 36.1	19	16
Iron (mg)	0.55- 0.65	0.4	-
Thiamine (mg)	0.28- 0.043	-	-
Riboflavin (mg)	0.043- 0.045	-	-
Niacin (mg)	1.297- 1.3	0.2	0.2
Ascorbic acid (mg)	8-9	25	4
Ash (g)	0.28	0.5	0.4

3.2.4 Chemical constituents in dragon fruit peel

Previous researches reported that both the flesh and peel of dragon fruit are very good sources of antioxidants such as polyphenolic compounds and betacyanin. In addition, they reported that dragon fruit peel showed a very high content of betacyanin pigment (**Table 3.6**) which is the major pigment found in dragon fruit peel. The antioxidant activity of the peel was even higher than that in the flesh. Besides, it was found that the chloroform extract from the peel could act as an antimicrobial substance by inhibiting the growth of food-borne pathogens. It is also a good source of dietary fiber such as pectin, lignin, and cellulose that are shown in **Table 3.7**. Interestingly, some research found that betacyanin content in red dragon fruit peel (*H. polyrhizus*) was higher when compared to the white dragon fruit peel (*H. undatus*) (43-46).

Table 3.6 Proximate composition and physico-chemical properties of dragon fruit peel, red flesh species (4)

Compositions/Properties	**Value**
Moisture (%)	92.65 ± 0.10
Protein (%)	0.95 ± 0.15
Fat (%)	0.10 ± 0.04
Ash (%)	0.10 ± 0.01
Carbohydrate (%)	6.20 ± 0.09
pH	5.06 ± 0.01
Titratable acidity (gl^{-1})	0.19 ± 0.04
Betacyanin (mg/100g dry matter)	**150.46 ± 2.19**
Organic acids concentration (%)	
Oxalic	0.80 ± 0.01
Citric	0.08 ± 0.00
Malic	0.64 ± 0.00
Succinic	0.19 ± 0.00
Fumaric	0.01 ± 0.00
Total acid	1.72

Table 3.7 Carbohydrate components of dragon fruit peel (4)

Carbohydrate components	**Percentage (%)**
Pectin	10.79 ± 0.01
Starch	11.07 ± 0.03
Cellulose	9.25 ± 1.33
Lignin	37.18 ± 1.02
Sugars	
Glucose	4.15 ± 0.03
Maltose	3.37 ± 0.01
Fructose	0.86 ± 0.02
Sucrose	ND*
Galactose	ND
Total sugar	8.38
Total dietary fiber	69.30 ± 0.53
Insoluble	56.50 ± 0.20
Soluble	14.82 ± 0.42

*ND: Not detected

3.2.5 Toxicity of dragon fruit peel

From previous studies, there have been no results demonstrating the toxicity of dragon fruit peel. It might be confirmed that dragon fruit peel extract is safe as enriched source of betalain and bioactive compounds.

Hor., *et al.* (2012) studied the toxicity of dosing a methanol extract of *H. polyrhizus* in Sprague-Dawley rats after single and 28 days repeated oral dosing. Single doses of extract, varied at 1250, 2500, and 5000 mg/kg, were given intravenously to rats and monitored for 14 days to study the acute toxicity. Furthermore, for the study on subchronic toxicity, they carried on the study using the same method but monitored for 28 days. Their results found that there were no mortality or signs of acute and subchronic toxicity which indicated by body weight, relative organ weight, and biochemical analysis. The No Observed Adverse Effect Level (NOAEL) of the extract for rats is 5000 mg/kg per day (47).

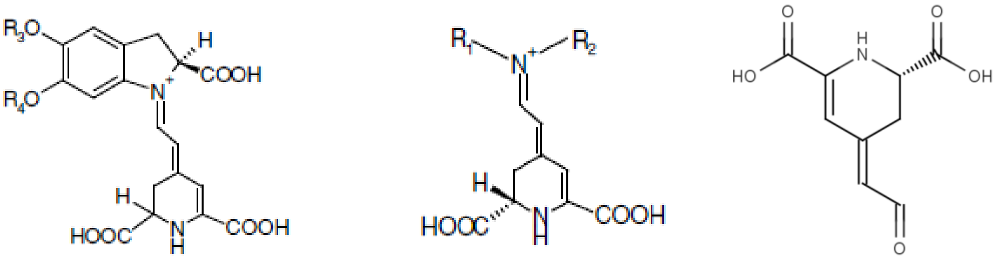
In the study of Shakir (2009), this study also found that *H. polyrhizus* extract did not produce the toxicity. Both of flesh and peel extracts were not toxic against embryonic normal liver cells and liver cancer cells. Moreover, the peel extract caused cell death in the liver cancer cells (48).

3.3 Betacyanin

Betalain has been used as a food colorant for centuries. At that time, people used the pokeberry juice to improve the color of red wine. Because of the high betalain content in beet root, it is the main commercial source of betalain at present. Betalain obtained from red beet root has been approved by US and EU legislations as natural colorant (**Table 3.1**). It was classified as vegetable juice that is spray-dried with maltodextrin to obtain the powder form. Other than beet root, there are several sources of betacyanin, for instance prickly pear, cactus pear, amaranth, and red dragon fruit. The advantage of betalain is not only in its use as a food colorant, but it also has strong antioxidant activities (2, 24, 49).

3.3.1 Characteristics of betacyanin

Betacyanin is a pigment classified under betalain (**Figure 3.9**). Betalain is a group of water-soluble nitrogen-containing pigments, which is synthesized from the amino acid tyrosine. It occurs in *Centrospermae*, especially in red beets, but also in some cactus fruits and mushrooms. The structure of betalain consists of betalamic acid, betanidin, and indicaxanthin. It might be that betalamic acid condenses with cyclo-dopa to yield betanidin which is the precursor aglycone of red betacyanin (6, 49). Thus betalain is divided into 2 structural groups: betacyanin (red-violet, λ_{max}~ 540 nm) and betaxanthin (yellow-orange, λ_{max}~ 480 nm). The major forms of betacyanin are betanin and glucoside of betanidin which account for 75-95% of total pigments of red beet. The major yellow pigments are vulgaxanthin I and II (50-52).



a b c

Figure 3.9 Structure of betalains; betacyanin (a), betaxanthin (b), betalamic acid (c) (2, 53)

3.3.2 Stability of betacyanin

The stability of the pigment is an important issue to be considered regarding its application. It is influenced by pH and heat, in particular. Betalain is relatively stable over a pH range of 3-7. An optimal pH range for the maximum color stability of betalain solution is between pH 5-6. For the temperature factor, some studies reported increasing betalain degradation rate with increasing temperature. During heat processing, the pigment will be degraded by isomerization or decarboxylation (51, 54). The mechanism is shown in **Figure 3.10**. Furthermore, betalain degradation is increased by light, UV or gamma radiation and oxygen. The factors affecting on the stability of betacyanin are presented in **Figure 3.11**. As mentioned, betacyanin is sensitive to many factors. Therefore, it is generally used in foods that have short shelf life, can avoid severe heating, light, and keep in the good package (2). Woo, *et al*. (2011) reported that keeping betalain pigment in a refrigerator at 4 °C in the dark could be preserve its color up to 3 weeks (7). A study found that some antioxidants such as ascorbic acid or isoascorbic acid can enhance the stability of betacyanin (30). In contrast, another study reported that ascorbic acid and citric acid could not reduce the color degradation (55). Thus, it could not be concluded that adding ascorbic acid would be helpful as a stabilizer for betacyanin.

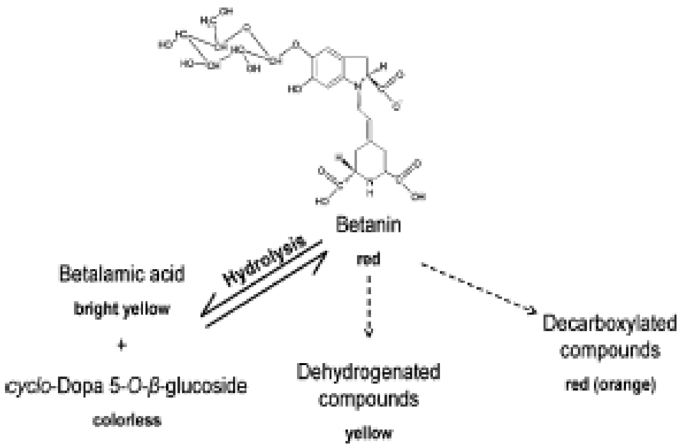


Figure 3.10 The initial step of thermal betanin degradation (56)

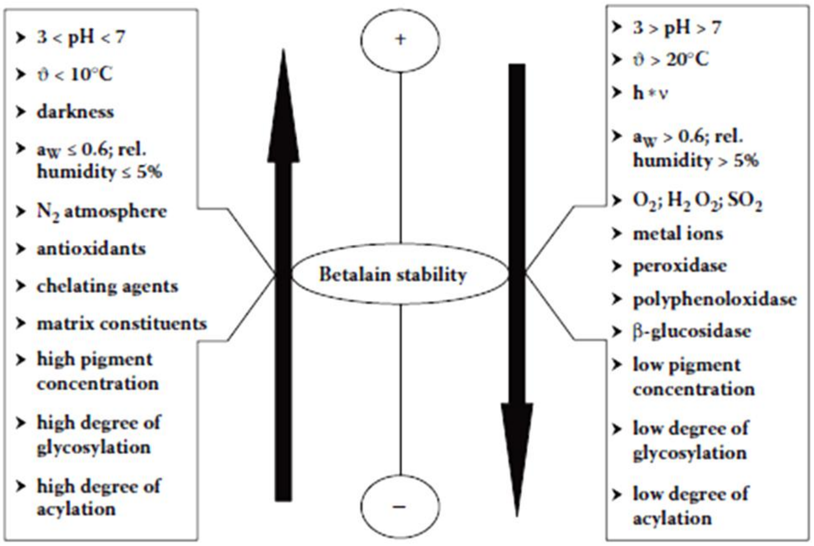


Figure 3.11 Factors affecting on the stability of betalain (6)

3.3.3 Extraction of betacyanin

Dragon fruit peel can be extracted using different kinds of solvent such as water, ethanol, or methanol (51). In an earlier research, extraction temperature was varied at room temperature, 40, 60, 80, and 100 °C for 10 min. They carried on the experiments by using the extracted solution. The researchers reported that using water at 100 °C was the best extraction solution to obtain the highest amount of pigment from dragon fruit. On the other hand, this condition changed the color from red-purple to scarlet red (57). Similar results were shown by Harivaindaran, *et al.* (2008). They found the optimal extraction condition was heating at 100 °C for 5 min (58).

In the study of Woo, *et al.* (2011), betacyanin pigment was prepared in the form of spray-dried pigment. Red dragon fruit was extracted using distilled water at the ratio 1:1 w/v and spray-dried (55). Naderi, *et al.* (2010) used *H. polyrhizus* for extraction with 50% ethanol at the ratio of 1:1 w/v of fruit flesh and solvent. After that, the mixture was centrifuged, filtered, and concentrated by using a rotary evaporator at 35 °C to obtain betacyanin concentrate as a food colorant. They improved the extraction yield by an enzymatic treatment to degrade the mucilage caused by pectin from its fruit. Color stability and overall betacyanin retention were studied (59). Other studies also mentioned about pectin in the fruit. Due to the high content of pectin in dragon fruit peel, it is necessary to precipitate the pectic substances after extraction by using ethanol (60-62).

Wybraniec, *et al.* (2001) studied the characteristics of betacyanin in *H. polyrhizus*. Dragon fruit was extracted using 80% ethanol at 1:2 w/v ratio of dragon fruit and solvent. The extraction step was carried out at room temperature and the extract was concentrated at 25 °C. The betacyanin concentrate was characterized by an HPLC method. The results exhibited 6 compounds of betacyanin which were betanin, isobetanin, phyllocactin, isophyllocactin, hylocerenin, and isohylocerenin (62). Phebe, *et al.* (2009) also followed the same extraction condition. They found that the total concentration of betanin and isobetanin in the peel was 8.72 mg/ml analyzed by an HPLC method (63).

Extraction with an unheated process was better for the color stability. Most extraction methods that used water as the extraction solvent would be followed by drying and spray-drying, or determination its properties was performed in form of the extracted solution. On the other hand, extraction by using ethanol was generally followed by evaporation to get rid of solvent. Moreover, using ethanol provided easier extraction and evaporation due to its lower boiling point than water.

3.4 Determination of betacyanin content

There are several methods to determine the betacyanin content. From the previous studies, spectrophometric absorption method and high-performance liquid chromatography (HPLC) were commonly reported.

3.4.1 Spectrophotometric absorption method

In many researches, determination of betacyanin content was performed by using a spectrophotometer and the pigment content was calculated as described below (28, 30, 59):

$$\text{Betacyanin content (mg/L)} = \frac{\text{A x DF x MW x 1000}}{(\varepsilon \text{ x L})}$$

Where A is the absorption value at 538 nm

DF is the dilution factor

MW is molecular weight of betacyanin (MW = 550 g/mol)

ε is the molar extinction coefficient of betacyanin (60,000 l/mol cm in H_2O)

L is the path length (1 cm) of the cuvette

When comparing the differences between the 2 methods, using spectrophometric absorption method appeared to have an important advantage. In most of the previous studies which determined betacyanin by using HPLC, mainly betacyanin was identified and quantified. The key limitation was that the standard compounds used were not extensive enough to represent all of the betacyanin pigments plus these standards are very expensive. Therefore, spectrophotometric measurement is preferred (64, 65) because this method is simpler and more feasible in terms of cost of analysis than using the HPLC method.

3.4.2 High-performance liquid chromatography method (HPLC)

Determination of betacyanin content by HPLC was used in several previous studies. There were differences in conditions for determination among the studies. Wybraniec and Mizrahi (2002) studied the betacyanin content in fruits of *Hylocereus* species by using HPLC. Trifluoroacetic acid and acetronitrile were used as the mobile phase solvents. Betacyanin was detected at 538 nm wavelength after separation through the C18 column (66). The condition was similar in other studies. However, in some studies formic acid and acetronitrile were used as the mobile phase solvents. Standard for betacyanin reference was the standard of betanin which is one subclass from various subclasses of betacyanin (65, 67).

3.5 Antioxidant activity

Antioxidant is defined as any substance which prolongs the shelf life of foodstuffs by protecting them against deterioration caused by oxidation such as fat rancidity and color changes (68).

Free radical is the molecules or fragments of molecules with at least one unpaired electron in the outer orbital (69).

3.5.1 Classification of antioxidants

3.5.1.1 Primary or chain-breaking antioxidants

Primary antioxidants are the antioxidants that terminate the chain of free radical formation by donating hydrogen of electrons to free radicals and stabilize the products. Examples of these antioxidants are tocopherols, gallates, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) (70).

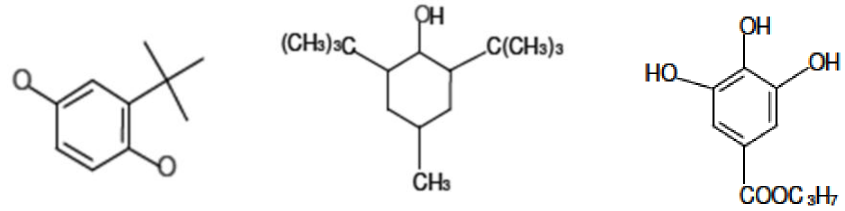
3.5.1.2 Secondary or preventive antioxidants

Secondary antioxidants are the antioxidants which function by scavenging free radicals before the oxidation chain begins such as peroxidase, catalase, and Co-Q_{10} (71)

3.5.2 Sources of food antioxidants

3.5.2.1 Synthetic antioxidants

BHA, BHT, tertiary-butyl hydroquinone (TBHQ), and propyl gallate (PG) are the commercial synthetic antioxidants. Different antioxidants have different properties depending on their chemical structure. These antioxidants have been approved by the food safety or food control authority in many countries. They have been used as food additives for a long time. However, most countries established an ADI for these antioxidants (68, 72) as a guideline for their usage. The chemical structures of some synthetic antioxidants are shown in **Figure 3.12**.

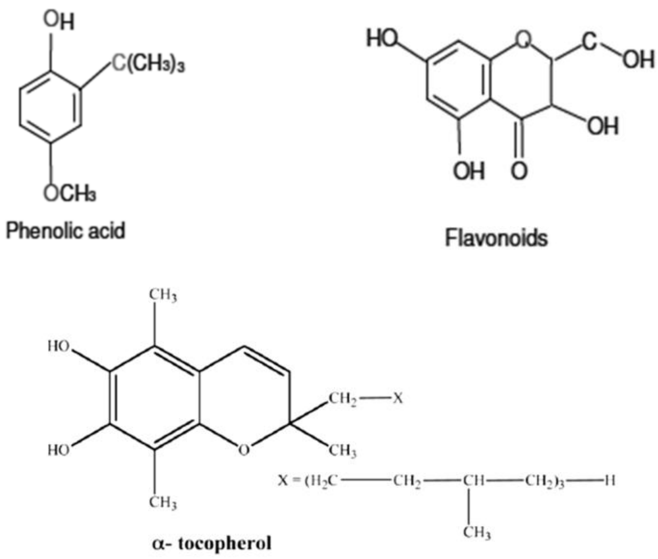


t-butylhydroquinone Butylated hydroxytoluene(BHT) Propylgallate(PG)

Figure 3.12 Some of synthetic antioxidants structures (73)

3.5.2.2 Natural antioxidants

Natural antioxidants occurred widely in animals and plants. Currently they have become an interesting issue for using in foods instead of the synthetic antioxidants due to the concern of consumers on synthetic chemicals. Some natural antioxidants such as tocopherols, phenolic acid, vitamin C, or lecithin are used in commercial food production. They not only prevent fat and oil deterioration, but also add an advantage to health benefits (72). The chemical structures of some natural antioxidants are shown in **Figure 3.13**.



Phenolic acid

Flavonoids

α- tocopherol

Figure 3.13 Structure of some natural antioxidants (73, 74)

3.5.3 Determination of antioxidant activity

There are many methods for determination of antioxidant activity such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, ferric-reducing antioxidant power assay (FRAP), ABTS radical cation decolorization assay, oxygen radical absorbance capacity (ORAC), and other methods. Normally, determination of antioxidant activity is carried out by 2 methods with different major mechanisms which are hydrogen atom transfer based method (HAT) and single electron transfer based method (SET).

HAT method measures the capacity of an antioxidant to terminate free radicals by hydrogen donation (AH: a hydrogen doner) (75).

$$X^{\bullet} + AH \longrightarrow XH + A^{\bullet}$$

SET method measures the capacity of the potential antioxidant to transfer one electron to reduce a compound including metals, carbonyls, and radicals (76).

$$X^{\bullet} + AH \longrightarrow X^{-} + AH^{\bullet +}$$
$$X^{-} + H_3O^{+} \longrightarrow XH + H_2O$$
$$M(III) + AH \longrightarrow AH^{+} + M(II)$$

Two different methods are usually selected for the determination of antioxidant activity. DPPH and ORAC were selected for determination method in this study.

3.5.3.1 DPPH assay

DPPH assay is based on single electron transfer based assay. This method is inexpensive, easy, fast, and widely used. It is a decolorization assay that determines the reduction activity of an antioxidant on DPPH radical. The DPPH radical absorbance is measured by the bleaching of purple to yellow or colorless at 517 nm. The DPPH value is calculated as the percentage of radical scavenging using the standard curve of trolox and expressed as μmole of trolox equivalent (μmol TE)/100 g sample (75, 77).

$$\underset{\text{purple}}{\text{DPPH}\bullet + \text{H–A}} \longrightarrow \underset{\text{yellow/colorless}}{\text{DPPH–H} + \text{A}\bullet}$$

3.5.3.2 ORAC assay

ORAC assay has been widely accepted as a standard method to determine the antioxidant activity in nutraceuticals, pharmaceuticals, and foods. Its condition is similar to the human body system. This method is a HAT-based method. It measures the ability of an antioxidant to inhibit peroxyl radical-induced oxidation. Peroxyl radical is formed from the breakdown of 2,2’-azobis (2-amidinopropane) dihydrochloride (AAPH). Then it can oxidize fluorescein to generate the product without fluorescence. Antioxidant inhibits this reaction by hydrogen atom transfer mechanism delaying the oxidative degradation of the fluorescein signal (**Figure 3.14**). The final ORAC activity is calculated using a linear regression equation between trolox concentration and the net area under curve (AUC) of standard (**Figure 3.15**). The net AUC is determined by subtracting the AUC of the blank from that of the sample. The results are expressed as μmole of trolox equivalent (μmol TE) per 100 g of sample (78-80).

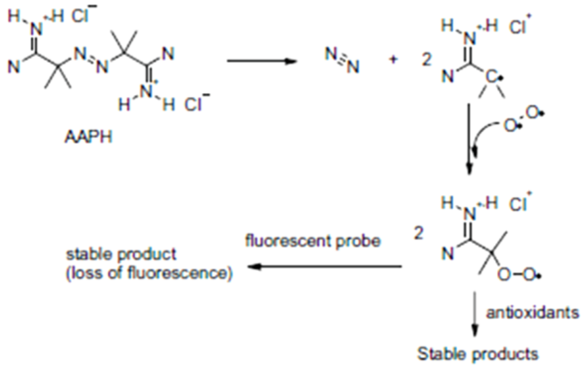


Figure 3.14 Reaction of the AAPH radical (81)

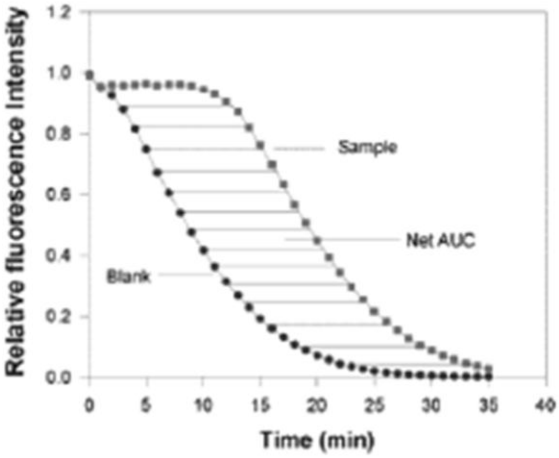


Figure 3.15 ORAC antioxidant activity of sample expressed as the AUC (75)

3.6 Color measurement systems in food

3.6.1 Munsell system

Munsell system is used as a standard color measurement system in the US and other countries. It is the oldest color measurement system. This system shows high consistency, no limitation by samples, rapid determination, and wide applications. The chromaticity of this system includes hue (H), value (V), and chroma (C). Color measurement by this system follows the Munsell books of color which are the reference guide books (**Figure 3.16**). It is the standards for color comparison. Each standard is associated with an alphanumeric notation. The letter assignment corresponds to major hue names such as yellow (Y), blue (B), green (G), purple-red (PR), etc. The value notation in front of the letter indicates the lightness. The value extends from 0 (absolute black) to 10 (absolute white). The chroma notation that comes after the letter indicates the degree of departure of a given hue from neutral gray of the same value. The value extends in equal step from 0 (neutral gray) to 10, 12, 14, or another. The color notation is shown “HV/C”, for instance 3Y4/10 (3Y: hue, 4: value, 10: chroma). (2, 82, 83).

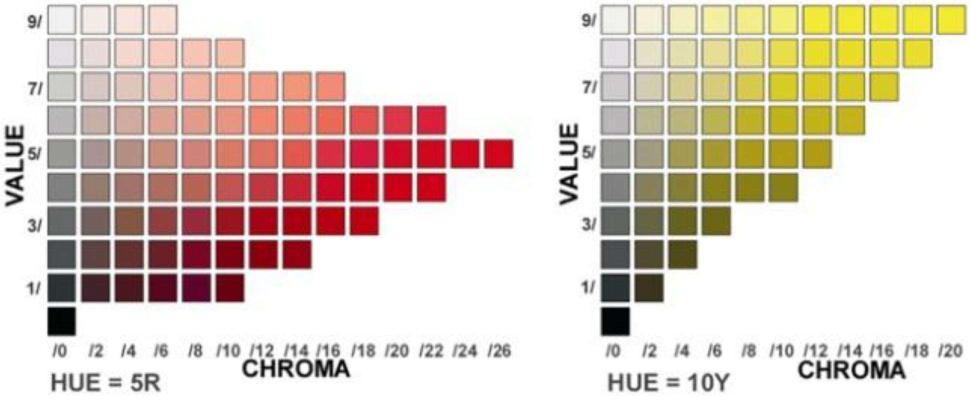


Figure 3.16 Samples of the color scheme page from the Munsell books of color (84)

3.6.2 CIE color systems

CIE (Internationale de I'Eclairage) means the International Commission on Ilumination. It is the body responsible for international recommendation on photometry and colorimetry. CIE color systems include 3 systems that are CIE XYZ, CIE L*a*b*, and CIE L*C*h°. The system CIE L*a*b* (**Figure 3.17**) is widely used in food color measurement. The L*, a*, and b* values represent lightness, redness/greenness, and yellowness/blueness, respectively. The L* value is expressed as 0-100 which is black to white. The a* value expressed by positive and negative values which represent redness to greenness. Finally, the b* value expressed by positive and negative values which represent yellowness to blueness (82, 83).

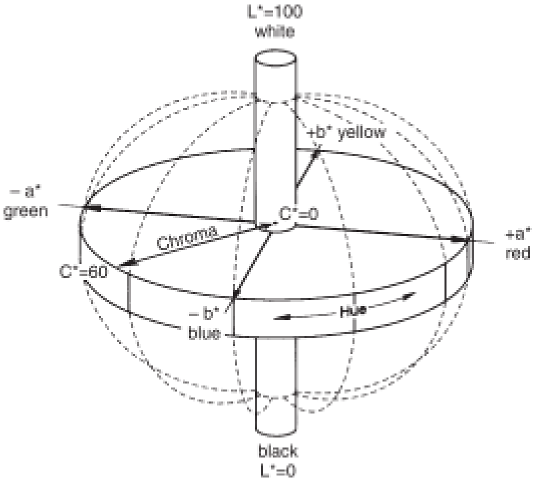


Figure 3.17 CIE L*a*b* color space (82)

CHAPTER IV
MATERIALS AND METHODS

4.1 Dragon fruit peel

Dragon fruit (*Hylocereus polyhizus*) peel was collected from the markets around Salaya, Nakhon Prathom area. It was used as a raw material for betacyanin extraction. Only the peel from the red flesh variety was used in the study.

4.2 Preparation of dragon fruit peel before extraction

Dragon fruit peel was cleaned and washed by passing through running tap water one time to remove the remaining flesh and foreign materials. After that dragon fruit peel was kept in a freezer at -20 °C until further use.

4.3 Extraction of betacyanin from dragon fruit peel

Frozen dragon fruit peel was thawed at 4°C overnight before extraction. It was then cut into the small pieces and blended using an electric blender. Dragon fruit peel was extracted following the method of Wybraniec *et al*. (2001) with a slight modification (62). Dragon fruit peel was extracted with 95% ethanol at a ratio of 1:2 (g of dragon fruit peel: ml of ethanol) for 10 min, followed by filtration using gauze fiber. The extract was concentrated in vacuo using a rotary evaporator at 40 ºC and adjusted to 60 ºC after 30 min. The extract was evaporated to make 100-fold betacyanin-rich concentrate (DF colorant). The betacyanin-rich aqueous solution was kept in an amber glass bottle and stored under refrigerated condition (4 °C) until used in further experiments.

4.4 Stability study of dragon fruit peel colorant

To study the stability of DF colorant, 5 ml of DF colorant was kept in amber glass bottles and stored at 4 °C for 2 months. The samples were taken by random sampling to determine the color properties (color parameters, betacyanin content, and antioxidant activity) at day 0, 3, week 1, 2, 4, 6, and 8. The methods of determination were described in section 4.6.

4.5 The effect of pH value on the stability of dragon fruit peel colorant

The effect of different pH values in the range of 3 to 7 on the shade of color, betacyanin content, and antioxidant activity of DF colorant was studied. An appropriate pH modifying solution (citric acid and sodium bicarbonate) was added into a 100-fold diluted solution of DF colorant to vary the pH value to 3, 5 and 7. Ten ml of adjusted sample were kept in amber glass bottles and stored at 4 °C. The color properties were determined during a storage time of 15 days. The samples were taken for analysis at day 0, 3, 6, 9, 12, and 15.

4.6 Analytical methods

The stability of DF colorant was determined by measurement of color, betacyanin content, and antioxidant activity during storage time.

4.6.1 Color measurement

DF colorant was diluted by 500 folds with deionized water for color measurement. The color value was measured by a colorimeter (Minolta CR-400, Konica Minolta Sensing, Inc., Japan). The color value was expressed as L*, a*, and b* which represent lightness, redness, and yellowness, respectively. All analyses were performed in triplicates.

4.6.2 Determination of betacyanin content by spectrophotometry

DF colorant was diluted by 400 folds with deionized water. Betacyanin content was determined using a UV–Vis spectrophotometer and calculated by applying the equation of Cai and Corke (1999) (30)

$$\text{Betacyanin content (mg/l)} = \frac{A \times DF \times MW \times 1000}{\varepsilon \times L} \quad (1)$$

Where A is the absorption value at 538 nm
DF is the dilution factor
MW is molecular weight of betacyanin (MW = 550 g/mol)
ε is the molar extinction coefficient of betacyanin
(60,000 l/mol cm in H_2O)
L is the path length (1 cm) of the cuvette (28)

4.6.3 Determination of antioxidant activities

Antioxidant activity was determined by two methods which are DPPH assay and ORAC assay.

4.6.3.1 DPPH free radical scavenging assay determination

DPPH free radical scavenging assay was carried out following the method of Binsan *et al*. (2008) with a slight modification (77). The extract was diluted with 95% ethanol in a proper dilution and 1 ml of sample extract was added with 2 ml of DPPH in 95% ethanol. The solution was mixed vigorously for 10 s and kept in the dark at room temperature for 30 min. The blank was 1 ml of ethanol mixed with 2 ml of DPPH reagent. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The radical scavenging activity was calculated and the results were expressed in term of μmole of trolox equivalent per ml of concentrated sample (μmol TE/ml). All analyses were performed in triplicates.

4.6.3.2 ORAC assay determination

Oxygen radical absorbance capacity (ORAC) was determined according to the method of Huang *et al*. (2002) with a slight modification (79). The extract was diluted with a working phosphate buffer solution at pH 7.2 in a proper dilution. A 500 μl aliquot of diluted sample was mixed with 3 ml of warm fluorescein working solution (37 ºC) in a cuvette with continuous mixing using a magnetic stirrer. After that 500 μl of 2,2’-azobis (2-amidinopropane) dihydrochloride (AAPH) was added into the mixed solution. The intensity of fluorescence was measured at 15 s intervals until reaching 5% intensity, using a spectrofluorometer with an excitation wavelength of 493 nm and an emission wavelength of 515 nm. The final ORAC activity was calculated using a linear regression equation between trolox concentration and the net area under curve (AUC) of standard. The AUC was calculated as follows:

$$\text{AUC} = (0.5+f_5/f_4+f_6/f_4+f_7/f_4+\ldots+f_i/f_4) \text{ x CT} \qquad (2)$$

Where f_4 is initial fluorescence reading at cycle 4

f_i is fluorescence reading at cycle i

CT is cycle time in minutes

The net AUC was determined by subtracting the AUC of the blank from that of the sample. The results were expressed as μmole of trolox equivalent per ml (μmol TE/ml) of concentrated sample.

4.7 Application of DF colorant in food products

Two food products (milk and jelly) were used as models to study the application of DF colorant. A synthetic colorant with a similar shade of color was selected for use as a comparison. The two food products have different ingredients and preparation process to show the varieties of applications in food products. Two food products were studied by sensory evaluation and storage test by color measurement and determination of antioxidant activities.

4.7.1 Preparation of pasteurized milk

Milk processing was separated into 4 batches. To study the effect of heat, the colorant was added at different steps before and after heating. The first two batches were added with DF colorant, and the other two batches were added with a comparable synthetic colorant (Erythrosine). Milk was heated in the water bath at 72 ºC for 15 s then immediately cooled down. The colorant was added to milk either before pasteurizing or into the pasteurized milk when the temperature of the milk was 45 ºC. After that, milk with added colorant was tested by sensory evaluation and storage test.

For storage test, 10 ml of pasteurized milk samples were kept in amber glass bottles and stored under refrigeration (4 °C) to study the stability of the added colorant for 1 week. The samples were taken at day 0, 1, 3, and 7 to determine the color parameter and antioxidant activities. The determination methods were given in section 4.6.

4.7.2 Preparation of jelly

For jelly, the ingredients were separated into two batches. The first batch was added with DF colorant and the other one was added with a comparable synthetic colorant (Erythrosine). The colorant was added into the jelly solution at 45 ºC. After that, 25 ml each of jelly solution were poured into small plastic cups, covered with lids, and waited until set. The cups of jelly were covered with aluminum foil to protect the light and kept in the refrigerator (4 °C) for 1 week. The samples were determined for the color parameters and antioxidants activities followed the methods in section 4.6.

4.8 Stability study of food products

4.8.1 Color measurement

The color of food samples were measured without dilution to compare the different color parameters (L*, a*, and b*) between food added with DF colorant and synthetic colorant by a colorimeter (Minolta CR-400, Konica Minolta Sensing, Inc., Japan).

4.8.2 Determination of antioxidant activities

Antioxidant activities of food products were determined by 2 methods as mentioned in section 4.6.3. Food product samples were prepared for the analysis by the methods as follows.

4.8.2.1 Preparation of milk samples

For DPPH assay, milk samples were diluted with 95% ethanol in the proper dilution and followed by the method explained in the section 4.6.3.1. After mixing of samples with DPPH solution, the mixed samples were centrifuged at 2612 xg (8000 rpm) for 7 min. The supernatant was measured for the absorbance. The milk samples needed to be centrifuged because the sedimentation of milk in 95% ethanol.

For ORAC assay, milk samples were diluted in the proper dilution with a working phosphate buffer solution at pH 7.2. The diluted samples were analyzed by following the method in section 4.6.3.2.

4.8.2.2 Preparation of jelly samples

For DPPH assay, 15 g of jelly were blended and centrifuged at 3900 xg (6000 rpm) for 15 min. The supernatant was used for the determination as described in section 4.6.3.1.

For ORAC assay, 5 g of jelly were blended and extracted using 5 ml of 70% ethanol followed by shaking at 100 rpm for 2 h. After that, the samples were centrifuged at 3900 xg (6000 rpm) for 15 min. The supernatant was used for the determination following the method in section 4.6.3.2.

4.8.3 Sensory evaluation

A sensory evaluation was performed to test the difference in acceptability of food products which were added with DF colorant and synthetic colorant. The samples were evaluated by 30 untrained panelists who are staff and graduate students at the Institute of Nutrition, Mahidol University, using 9-point hedonic scales for preference test and 15-cm line scales for intensity test. The meaning of sensory properties was explained to all panelists for color and odor acceptance (score 1= dislike extremely, score 5 = neither like nor dislike and score 9= like extremely).

The samples were prepared one day before evaluation, packed and stored in the refrigerator (4 °C). Each sample was served in a clear plastic cup coded with a three-digit random number. The samples were served randomly in sets of samples on a plate to compare the differences. The evaluation was performed in air-conditioned testing booths under a daylight lamp at a sensory laboratory of the Institute of Nutrition, Mahidol University.

4.9 Statistical analysis

The data were analyzed with SPSS statistical software version 16.0 to distinguish significant differences among groups at 5% level of probability. The results were expressed as mean ± SD. All experiments were performed in triplicates. Significant differences between groups were considered when $p \leq 0.05$. The mean difference was compared using the Duncan's Multiple Range test.

One-way ANOVA was used to analyze the data groups of sensory evaluation of milk, color parameters, betacyanin content, and antioxidant activities during storage. After that, Duncan's Multiple Range test was used with significant difference ($p \leq 0.05$) to compare mean differences.

Two data groups of jelly sensory scores (synthetic and DF colorant added jelly) were analyzed by using T-test.

CHAPTER V

RESULTS

5.1 Appearance of dragon fruit peel colorant

Betacyanin-rich concentrate (DF colorant) extracted from dragon fruit peel (red flesh) was dissolved in deionized water. It exhibited dark red color. The photograph of DF colorant is shown in **Figure 5.1**.

(a) (b)

Figure 5.1 Dragon fruit peel colorant extracted from dragon fruit peel; (a) colorant in bulk solution and (b) one drop of colorant

5.2 Stability of dragon fruit peel colorant

The stability of DF colorant was studied by determination of color parameters, betacyanin content, and antioxidant activities.

5.2.1 Color parameters

The change in color parameters of DF colorant during storage at 4 °C for 8 weeks is displayed in **Figure 5.2**.

For L* value (lightness): The lightness values of DF colorant during storage time were in the range of 45-52 with a significant difference ($p \leq 0.05$) noted during the storage period. The values markedly increased after 14 days and showed an increasing trend during storage time for 8 weeks.

For a* value (greenness-redness): The a* values (+a = redness) of DF colorant during storage time were in the range of 22-7 which correspond to red color. They showed a decreasing trend after storage for 1 week with a significant difference ($p \leq 0.05$).

For b* value (blueness-yellowness): The b* values of DF colorant during storage time were in the range of (-0.6)-5. An increasing trend of b* values was observed with a significant difference ($p \leq 0.05$). The b* value increased after storage for 3 days which correspond to yellow color.

From all results of color parameters, DF colorant was rather unstable. The differences of color parameters were found within 1-2 weeks of storage. The color faded which could be observed by visual observation. However, the color of DF colorant was still in the same shade as shown in **Figure 5.2**. It was noted that if the higher amount of colorant was used the similar color intensity could possibly be maintained. The results and data of color parameters measurement are presented in **Figure 5.3** and **Appendix C**.

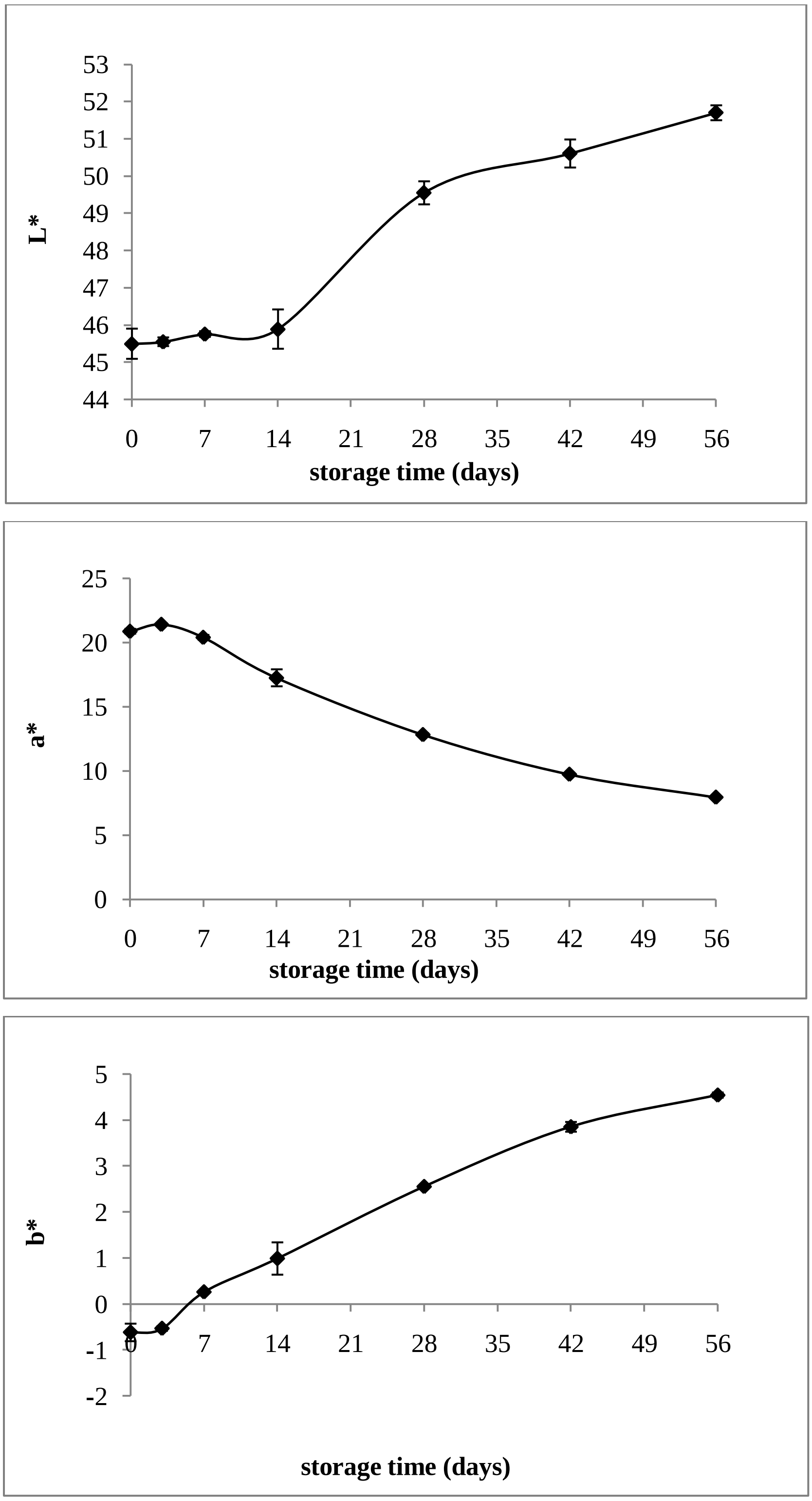


Figure 5.2 L* a* b* color parameters of DF colorant during storage at 4 °C for 8 weeks

Figure 5.3 Five hundred-fold diluted DF colorant during storage at 4 °C for 8 weeks

5.2.2 Betacyanin content

Betacyanin content of DF colorant was determined by spectrophotometry method. The result and data are shown in **Figure 5.4** and **Appendix D**.

The average betacyanin content on day 0 of storage was 1.25±0.01 mg/ml. The decreasing trend of betacyanin content was observed during storage for 8 weeks with significant difference ($p \leq 0.05$). At first week of storage, betacyanin content was in range of 1.22-1.25 mg/ml. After that the content decreased slightly to 0.61 mg/ml at the final day of storage ($p \leq 0.05$). The results correspond well with the results of color change as described in 5.2.1.

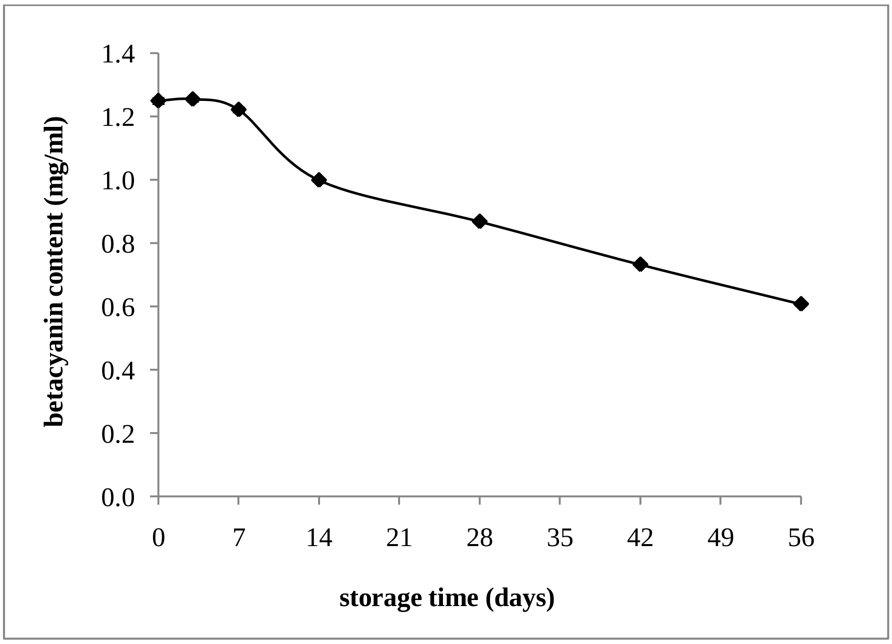


Figure 5.4 Betacyanin content in DF colorant during storage at 4 °C for 8 weeks

5.2.3 Antioxidant activities

The antioxidant activities of DF colorant were determined by DPPH assay and ORAC assay.

5.2.3.1 DPPH free radical scavenging assay

The results of antioxidant activities determined by DPPH assay are illustrated in **Figure 5.5** and **Appendix E**. At day 0 of storage, the antioxidant activity of DF colorant was 19.37±0.08 μmol TE/ml. The antioxidant activities decreased immediately after first day (day 0) of storage with a significant difference ($p \leq 0.05$). They decreased slightly from 19.37±0.08 μmol TE/ml to 12.77±0.25 μmol TE/ml at the last day of storage.

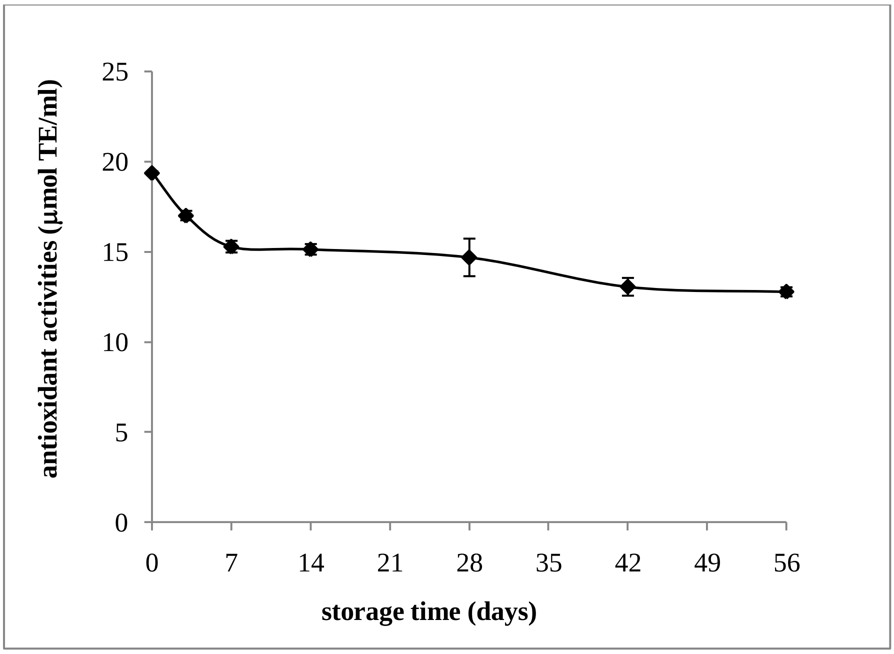


Figure 5.5 Antioxidant activities of DF colorant determined by DPPH assay during storage at 4 °C for 8 weeks

5.2.3.2 ORAC assay

The average antioxidant activities determined by ORAC assay was 222.18±25.85 μmol TE/ml at day 0 of storage. The antioxidant activity increased slightly at day 3 of storage. After that the decreasing trend was found during storage time with a significant difference ($p \leq 0.05$). The antioxidant activities decreased to 147.72±8.14 μmol TE/ml. All results of antioxidant activities during storage for 8 weeks are shown in **Figure 5.6** and all data are included in **Appendix F**.

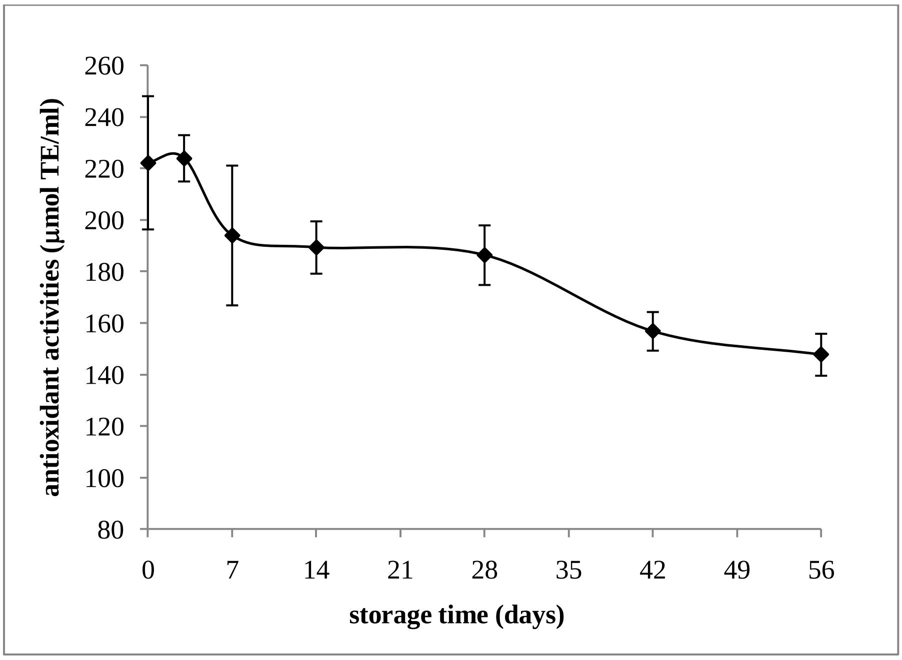


Figure 5.6 Antioxidant activities of DF colorant determined by ORAC assay during storage at 4 °C for 8 weeks

5.3 Effect of pH value on the stability of dragon fruit peel colorant

The stability of DF colorant as affected by the pH value was studied by determination of color parameters, betacyanin content, and antioxidant activities during storage time for 15 days.

5.3.1 Color parameters

The results of the effect of pH on the color values of DF colorant are shown in **Figure 5.7** and **Appendix G**.

For L* value (lightness): The values of all DF colorant were in the range of 46 to 53 with significant differences ($p \leq 0.05$) among the samples at different pH values. The L* values of DF colorant at pH 3 and 5 showed a slight increasing trend during storage. On the other hand, the results of L* for the sample at pH 7 showed fluctuated values ($p \leq 0.05$). They decreased after day 0 until day 6 of storage then increased after day 6 and decreased again after day 9 of storage.

For a* value (greenness-redness): The values of all DF colorant were in the range of 12 to 24 with significant differences ($p \leq 0.05$) among the samples at different pH values. DF colorant at pH 5 showed the highest a* value. The a* values at pH 7 gave fluctuated results which were similar to the results of L* values.

For b* value (blueness-yellowness): The values of all DF colorant were in the range of (-7) to (-3) with significant differences ($p \leq 0.05$) among the samples at different pH values. The results showed the lowest b* values in DF colorant at pH 5. The b* values of DF colorant at pH 7 also behaved in the same way with the L* and a* value.

From all results, the differences between DF colorant at pH 3, 5, and 7 were obvious. However, little changes of color parameters were found during storage for 15 days. Moreover, the shades of DF colorant among different pH values were not clearly distinguishable by visual observation especially at day 0 of storage (Figure 5.8). DF colorant at pH 5 exhibited the highest stability when compared to other pH values after storage for 15 days.

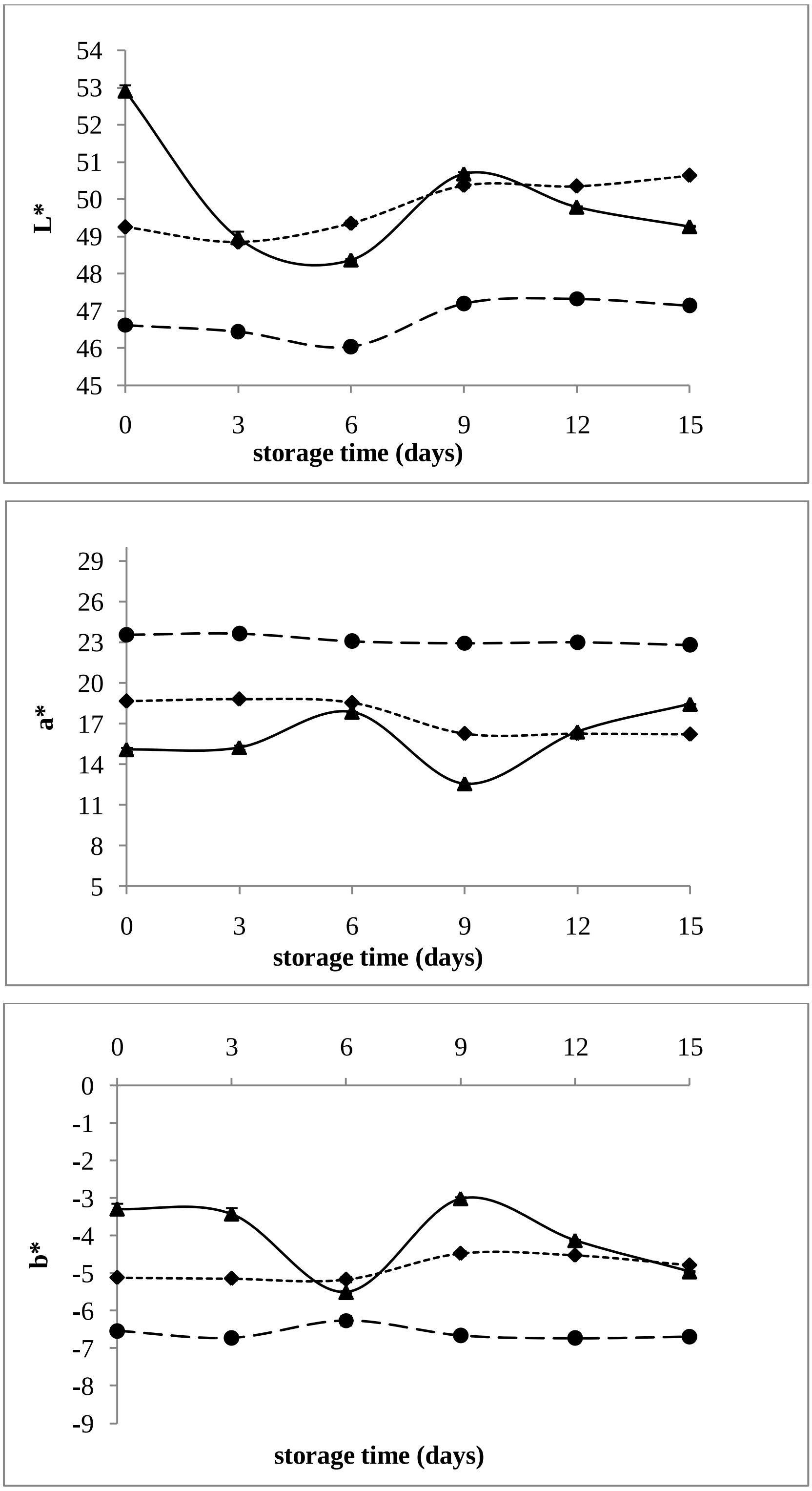


Figure 5.7 L* a* b* color parameters of DF colorant at different pH values during storage at 4 °C for 15 days (---◆--- pH 3, –●– pH 5, and —▲— pH 7)

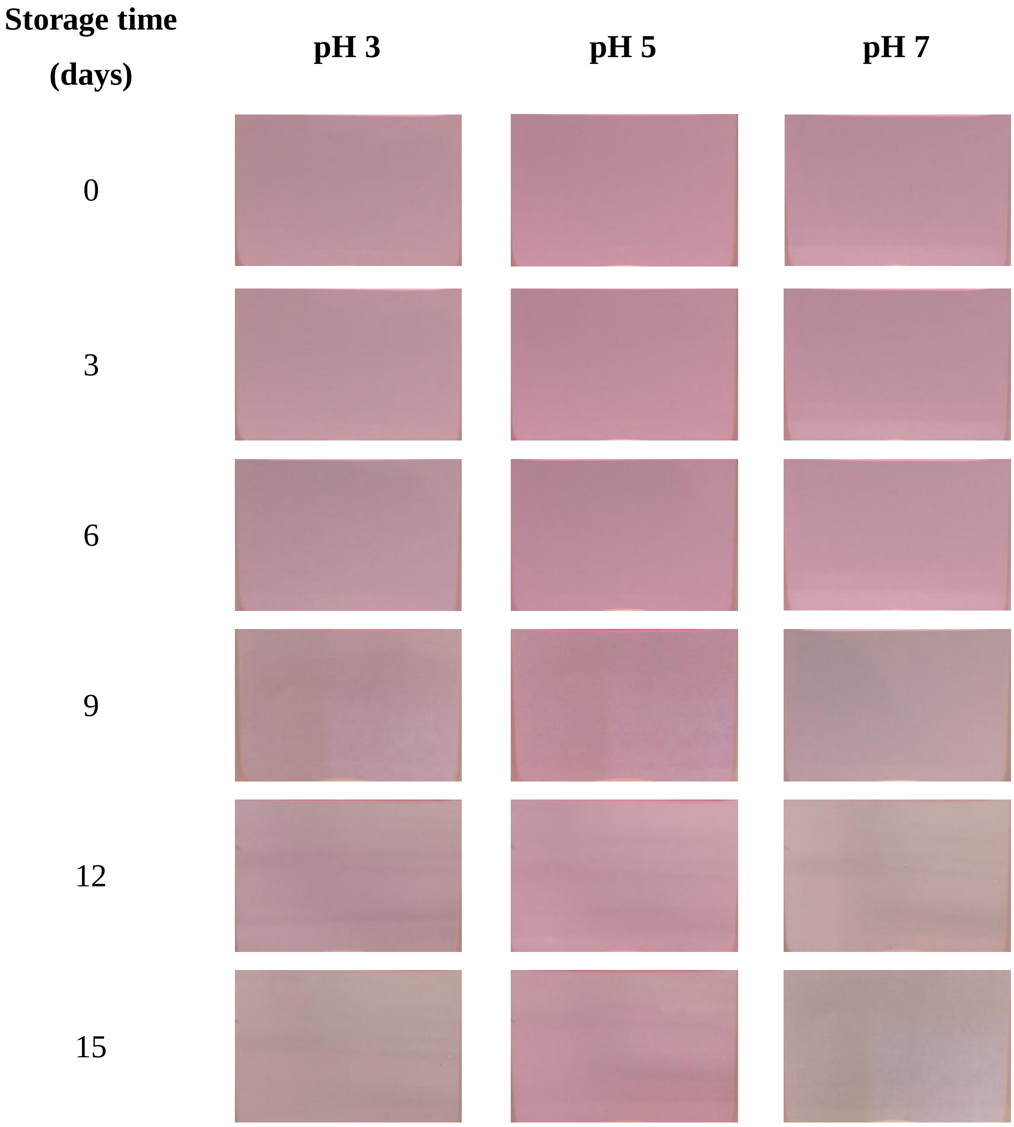


Figure 5.8 Five hundred-fold diluted DF colorant at different pH values during storage at 4 °C for 15 days

5.3.2 Betacyanin content

The results of betacyanin content analyzed by spectrophotometry method are illustrated in **Figure 5.9** and the data are included in **Appendix H**. At the first day of storage, the average betacyanin content in DF colorant at pH 3, 5, and 7 were 1.34±0.00, 2.27±0.01, and 1.63±0.01 mg/ml in concentrated sample, respectively. There were significant differences among the samples at different pH values ($p \leq 0.05$). The results showed DF colorant at pH 5 had the highest betacyanin content, followed by the samples at pH 7 and 3. Betacyanin content of different pH value tended to increase after day 0 of storage until day 3 of storage with significant difference ($p \leq 0.05$). After that betacyanin content tended to decrease slightly until the last day of storage ($p \leq 0.05$). These results agree very well with the results of color parameters measurement in DF colorant described in section 5.3.1.

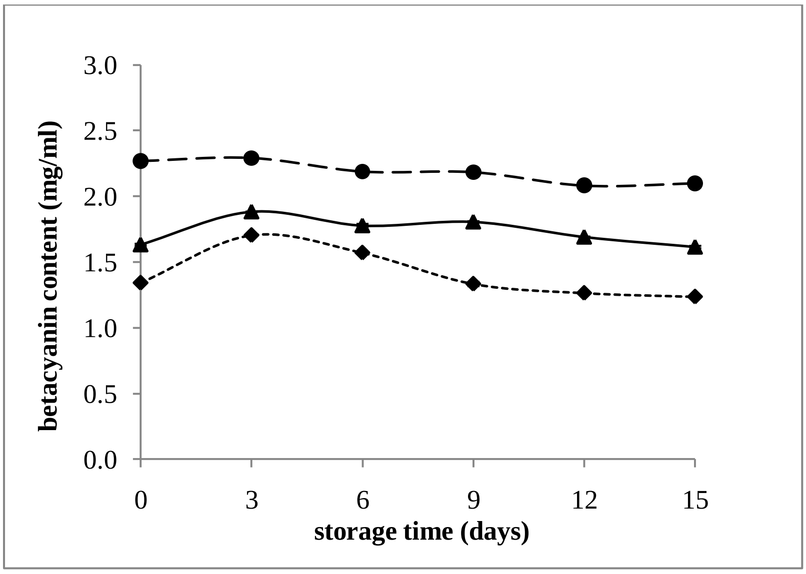


Figure 5.9 Betacyanin content of DF colorant at different pH values during storage at 4 °C for 15 days (---◆--- pH 3, –●– pH 5, and —▲— pH 7)

5.3.3 Antioxidant activities

5.3.3.1 DPPH free radical scavenging assay

The results of DPPH antioxidant activities analyses of the samples adjusted to pH 3, 5, and 7 are shown in **Figure 5.10** and **Appendix I**. Antioxidant activities of the samples adjusted to pH 3 and 5 were not significantly different (p>0.05). At day 0 of storage, the average antioxidant activities of those samples were 23.24±0.09 and 22.88±0.41 μmol TE/ml, respectively. In contrast, the sample adjusted to pH 7 presented the lowest antioxidant activity (8.24±0.55 μmol TE/ml) when compared to other samples (p≤0.05). All DF colorant samples indicated decreasing trend in antioxidant activities after storage at 4 ºC for 15 days.

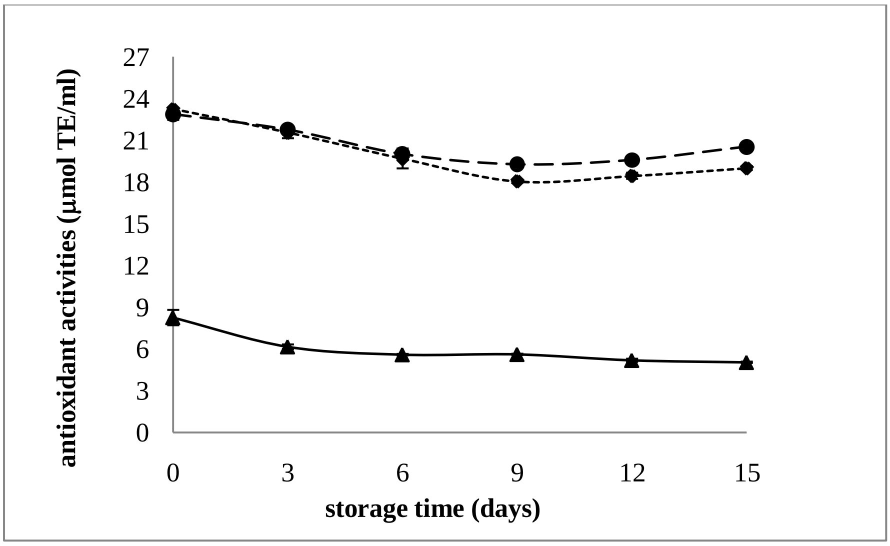


Figure 5.10 Antioxidant activities of DF colorant at different pH values determined by DPPH assay during storage at 4 °C for 15 days (--◆-- pH 3, –●– pH 5, and —▲— pH 7)

5.3.3.2 ORAC assay

Antioxidant activities of all DF colorant by ORAC assay indicated significant differences among samples ($p \leq 0.05$). The sample at pH 5 showed the highest antioxidant activity (203.49±12.75 μmol TE/ml) whereas the sample at pH 7 showed the lowest antioxidant activity (154.87±10.70 μmol TE/ml). The average antioxidant activities of the sample adjusted to pH 3 was 180.20±10.78 μmol TE/ml. Antioxidant activities of all samples tended to decrease with a significant difference ($p \leq 0.05$) during storage time for 15 days. The sample at pH 7 showed a decrease in antioxidant activity after day 6, while a decrease in antioxidant activity at pH 3 and 5 occurred after day 12 of storage time. The results are shown in **Figure 5.11** and **Appendix J**.

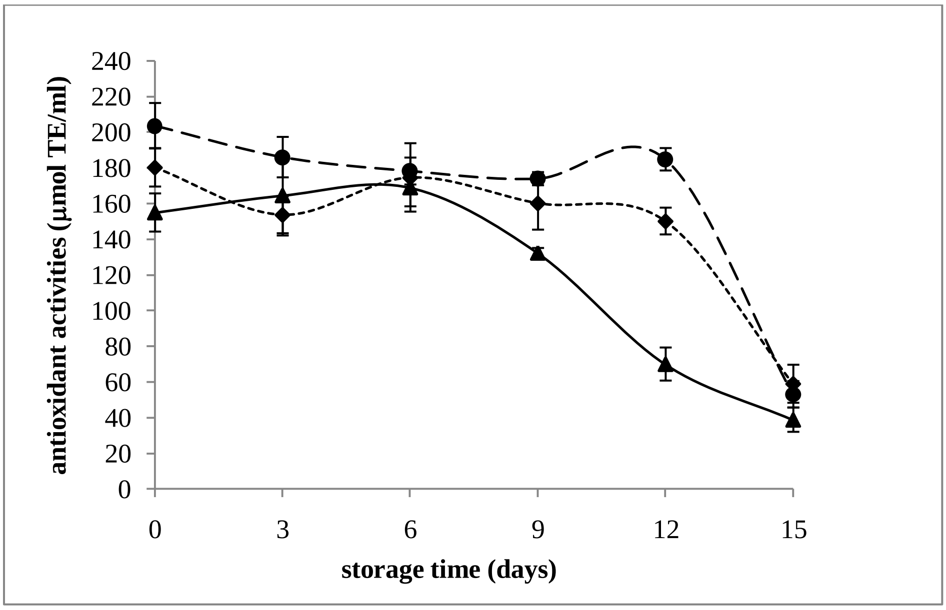


Figure 5.11 Antioxidant activities of DF colorant at different pH values determined by ORAC assay during storage at 4 °C for 15 days (--◆-- pH 3, –●– pH 5, and —▲— pH 7)

5.4 Application and stability in food products

5.4.1 Pasteurized milk

5.4.1.1 Color parameters

The results of color parameters are shown in **Figure 5.12** and all data are included in **Appendix K**. The color shade of pasteurized milk added colorant is illustrated in **Figure 5.13**.

For L* value (lightness): The values of synthetic colorant added milk and DF colorant added milk were significantly different ($p \leq 0.05$). The L* value of synthetic colorant added milks showed a higher value than those of milk added with DF colorant. DF colorant-added milk samples showed a slight increasing trend in L* value during storage. Moreover, the samples added with DF colorant before (dfah) and after (dfha) heating were found significantly different ($p \leq 0.05$). The average L* value of those samples was 68.08±0.03 and 67.39±0.06, respectively at day 0 of storage. While L* value of the samples added with synthetic colorant before (sah; 69.97±0.01) and after (sha; 70.23±0.01) heating were not significantly different ($p > 0.05$).

For a* value (greenness-redness): The a* value of samples at day 0 of storage was in the range of 10 to 13 with a significant difference ($p \leq 0.05$). The sample added DF colorant after heating (dfah) showed the least redness (10.79±0.01). The samples added DF colorant before and after heating found a significant decrease of redness after day 3 of storage time ($p \leq 0.05$).

For b* value (blueness-yellowness): The average b* values of all samples at day 0 of storage were in the range of 1.5 to 2.4 with a significant difference ($p \leq 0.05$). The values corresponded to yellow color. The sample added with DF colorant before heating indicated the highest yellowness (2.32±0.02) since the first day of storage. There was an increase in yellowness in both of dfah and dfha samples during storage time for 1 week ($p \leq 0.05$).

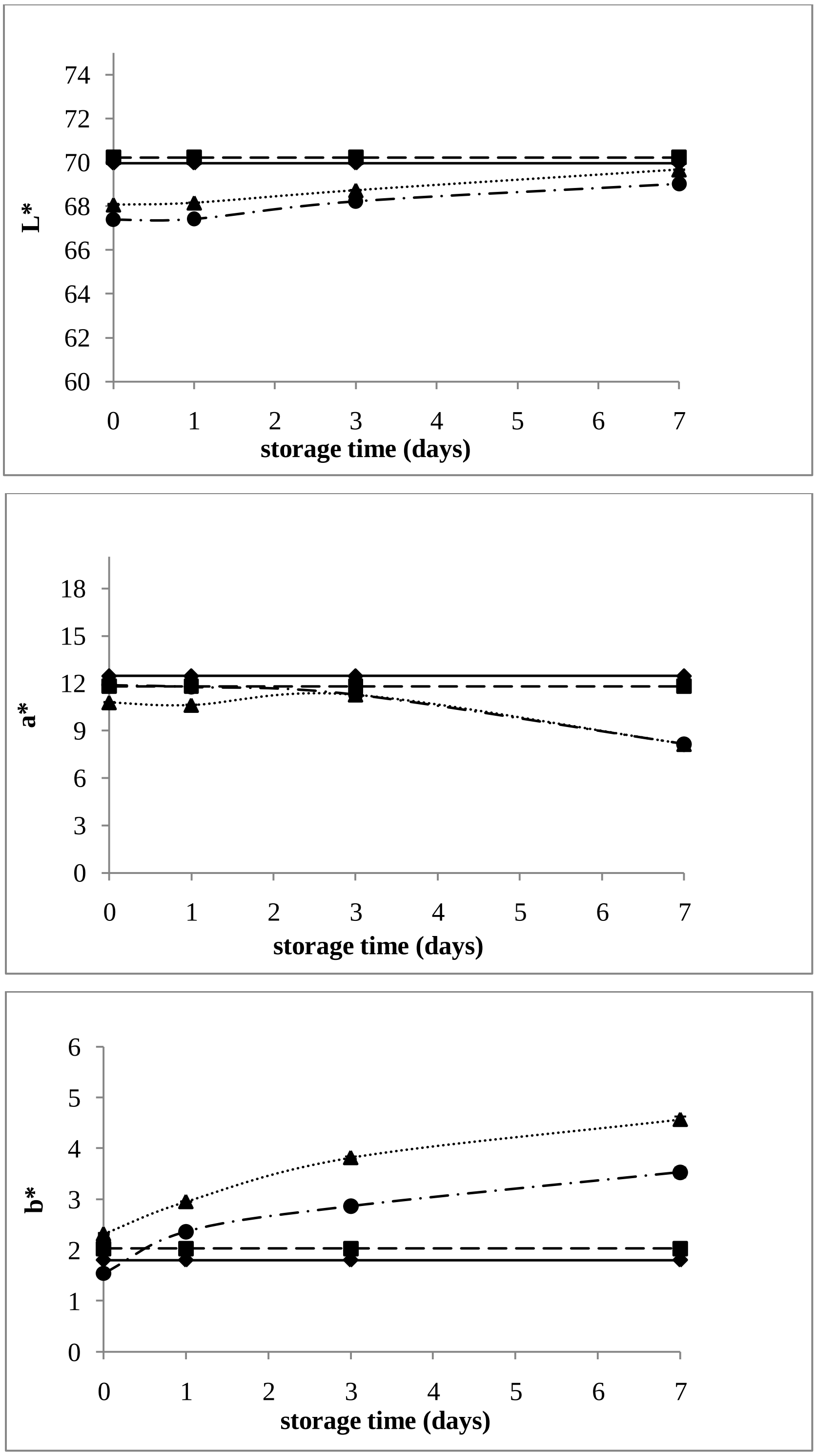


Figure 5.12 L* a* b* color parameters of colorant-added pasteurized milk during storage at 4 °C for 1 week (—◆— sah; milk added synthetic colorant before heating, –■– sha; milk added synthetic colorant after heating, ·····▲····· dfah; milk added DF colorant before heating, –●- dfha; milk added DF colorant after heating)

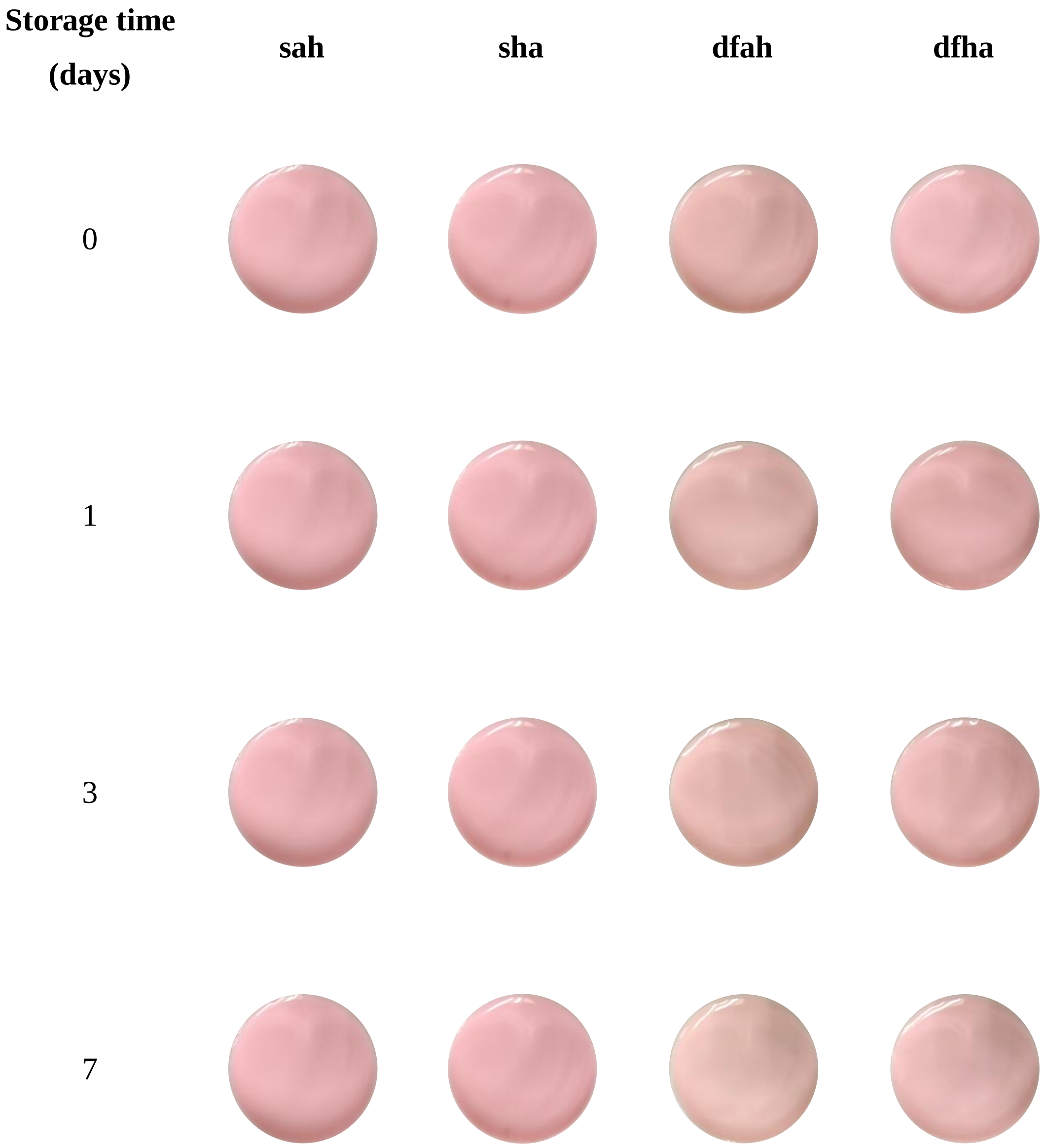


Figure 5.13 Colorant-added pasteurized milk during storage at 4 °C for 1 week

5.4.1.2 Antioxidant activities

For DPPH assay: The results of antioxidant activities determined by DPPH assay are presented in **Figure 5.14** and **Appendix L**. At day 0 of storage, the antioxidant activities of all products were in range of 0.17-0.21 μmol TE/ml with a significant difference ($p \leq 0.05$). The sample added with DF colorant after heating exhibited the highest antioxidant activities (0.21±0.02 μmol TE/ml). The antioxidant activities of both dfah and dfha slightly increased during the first day of storage ($p \leq 0.05$). Then they decreased after stored for 1 day with a significant difference ($p \leq 0.05$).

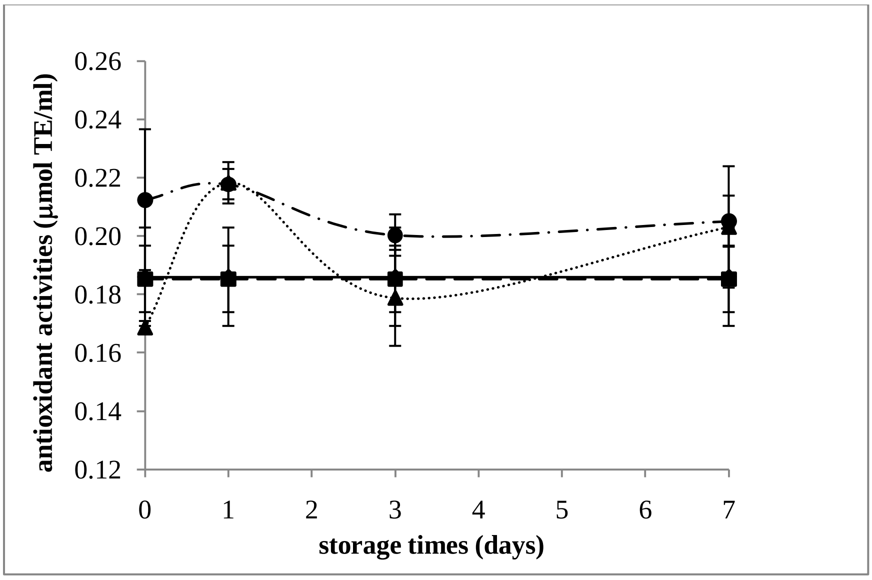


Figure 5.14 Antioxidant activities of colorant-added pasteurized milk determined by DPPH assay during storage at 4 °C for 1 week (—◆— sah; milk added synthetic colorant before heating, –■– sha; milk added synthetic colorant after heating, ·····▲····· dfah; milk added DF colorant before heating, – ● - dfha; milk added DF colorant after heating)

For ORAC assay: At the first day of storage, the average antioxidant activities determined by ORAC assay (**Figure 5.15** and **Appendix M**) were in the range of 24-27 μmol TE/ml. After stored for 1 day, antioxidant activities of milk added with DF colorant before and after heating increased from 18.89±0.57 to 33.11±3.36 μmol TE/ml and 26.27±0.77 to 43.14±0.90 μmol TE/ml, respectively ($p \leq 0.05$). They tended to decrease after 1 day of storage. The sample added with DF colorant after heating exhibited the highest antioxidant activities with a significant difference ($p \leq 0.05$) from others.

From the results, the antioxidant activities determined by DPPH and ORAC assay agreed well with each other. Both methods indicated a similar trend of antioxidant activities in the samples. DF colorant-added milk indicated higher antioxidant activities than those found in synthetic colorant-added milk. Moreover, milk added with DF colorant after heating showed the highest antioxidant activities with a significant difference ($p \leq 0.05$).

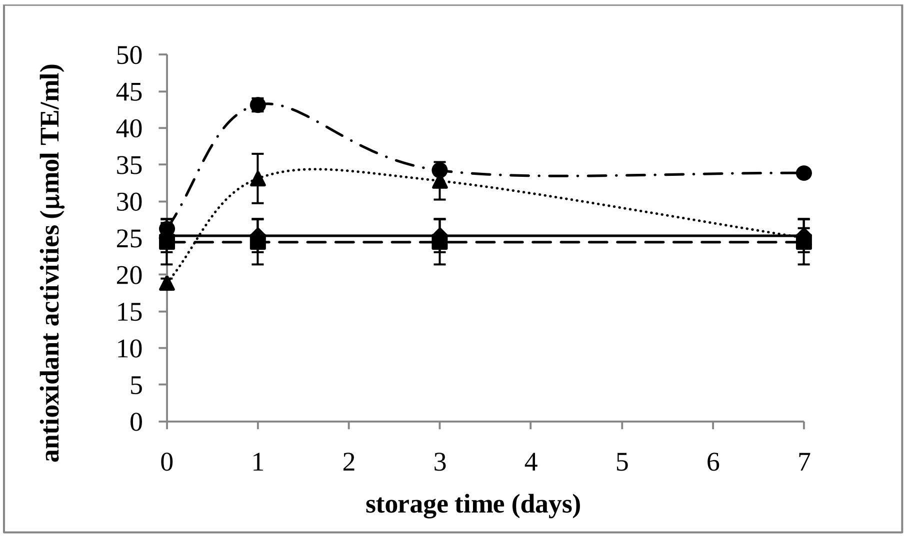


Figure 5.15 Antioxidant activities of colorant-added pasteurized milk determined by ORAC assay during storage at 4 °C for 1 week (—◆— sah; milk added synthetic colorant before heating, –■– sha; milk added synthetic colorant after heating, ····▲···· dfah; milk added DF colorant before heating, –●– dfha; milk added DF colorant after heating)

5.4.2 Jelly

5.4.2.1 Color parameters

All results of colorant-added jelly are shown in **Figure 5.16**, and the data are included in **Appendix N**. Furthermore, the color differences of samples could be noted by visual observation. The photographs of jelly were illustrated in **Figure 5.17** to compare the differences among samples and during storage time for 1 week.

For L* value (lightness): The values of synthetic colorant-added jelly (30.85±2.02) and DF colorant-added jelly (30.29±1.69) were not significantly different ($p>0.05$). The L* value of DF colorant-added jelly increased at day 1 of storage. Then it decreased slightly during further storage.

For a* value (greenness-redness): The a* values were not significantly different ($p>0.05$) between synthetic colorant-added jelly and DF colorant-added jelly at the first day of storage (12.23±1.06 and 9.99±1.05, respectively). The redness of DF colorant added jelly decreased slightly after day 1 of storage and tended to increase after that with a significant difference ($p\leq0.05$).

For b* value (blueness-yellowness): The b* values of synthetic colorant-added jelly (1.38±0.19) and DF colorant-added jelly (-0.19±0.13) were significantly different ($p\leq0.05$). DF colorant-added jelly exhibited a small fluctuation in the b* values during the storage period. Nevertheless, the values were not significantly different ($p>0.05$).

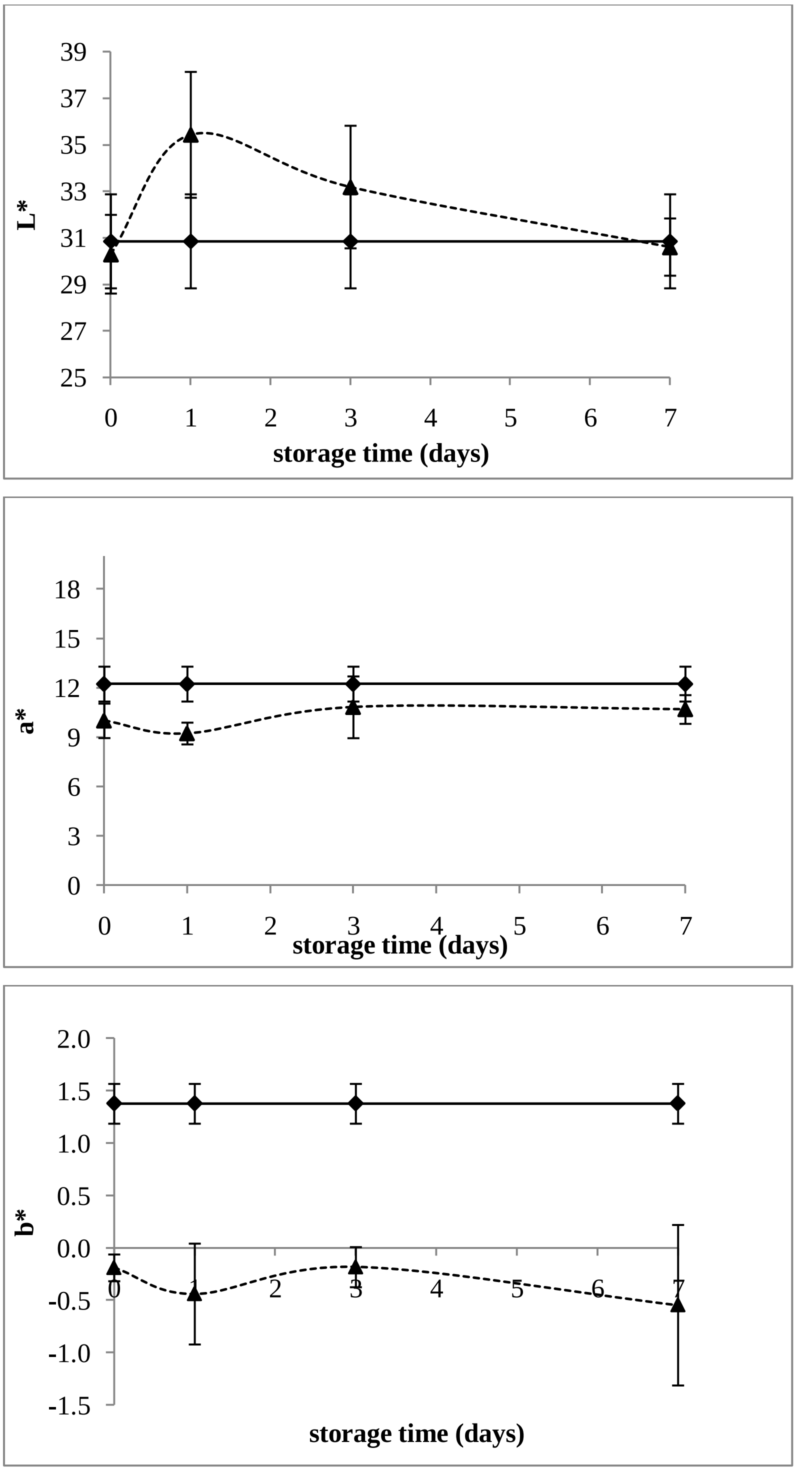


Figure 5.16 L* a* b* color parameters of colorant-added jelly during storage at 4 °C for 1 week (—◆— syn; synthetic colorant-added jelly and ---▲---DF colorant-added jelly)

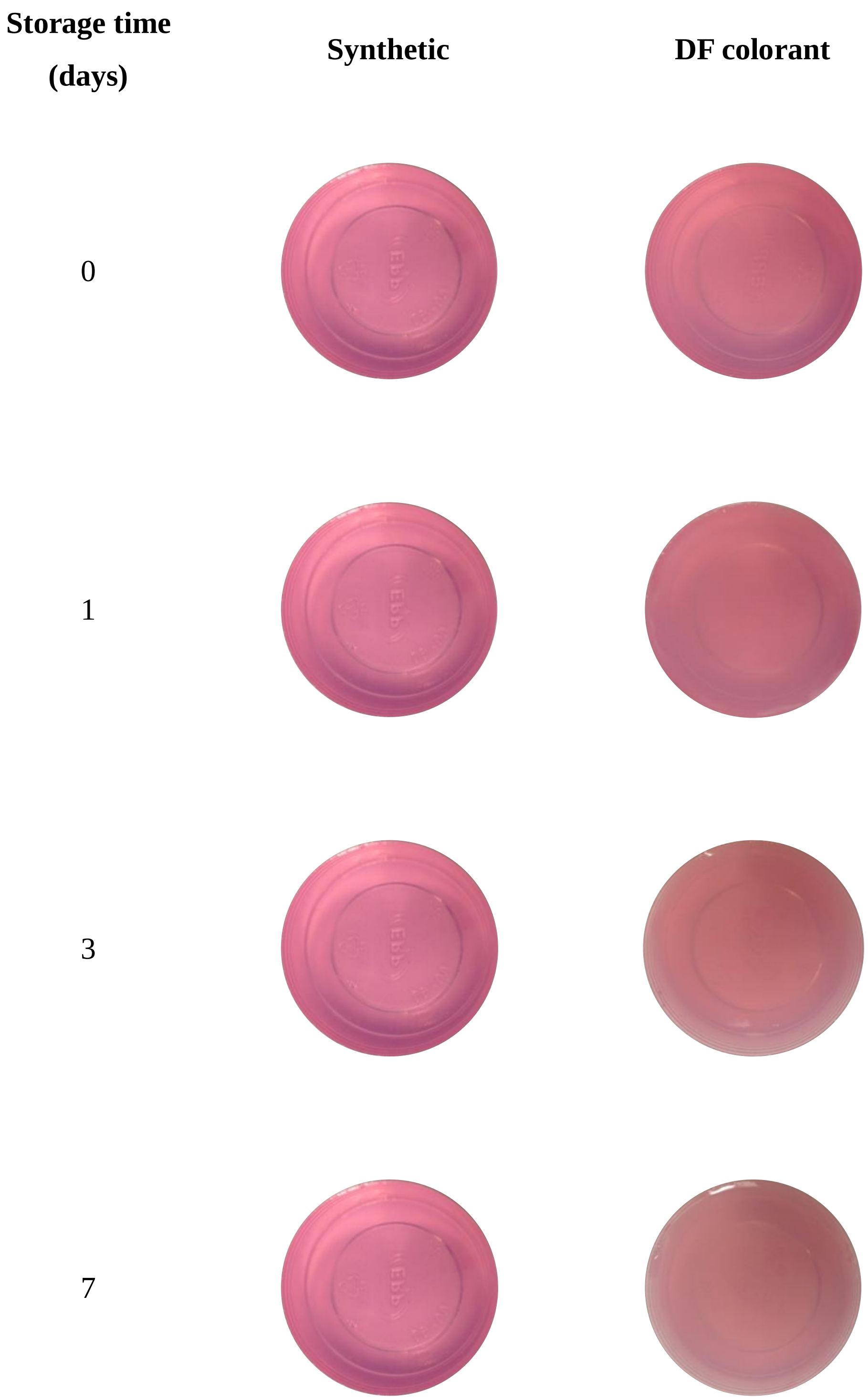


Figure 5.17 Colorant-added jelly during storage at 4 °C for 1 week

5.4.2.2 Antioxidant activities

For DPPH assay: The results of antioxidant activities determined by DPPH assay are presented in **Figure 5.18** and **Appendix O**. Antioxidant activities of synthetic and DF colorant-added jelly were significantly different ($p \leq 0.05$). The average antioxidant activities of those samples were 0.05±0.01 and 0.17±0.01 μmol TE/g, respectively at the first day of storage (day 0). After the first day of storage, antioxidant activities of DF colorant-added jelly tended to increase with a significant difference ($p \leq 0.05$). It was highest at day 3 of storage (0.35±0.01 μmol TE/g) and then tended to decrease during the remaining period of storage.

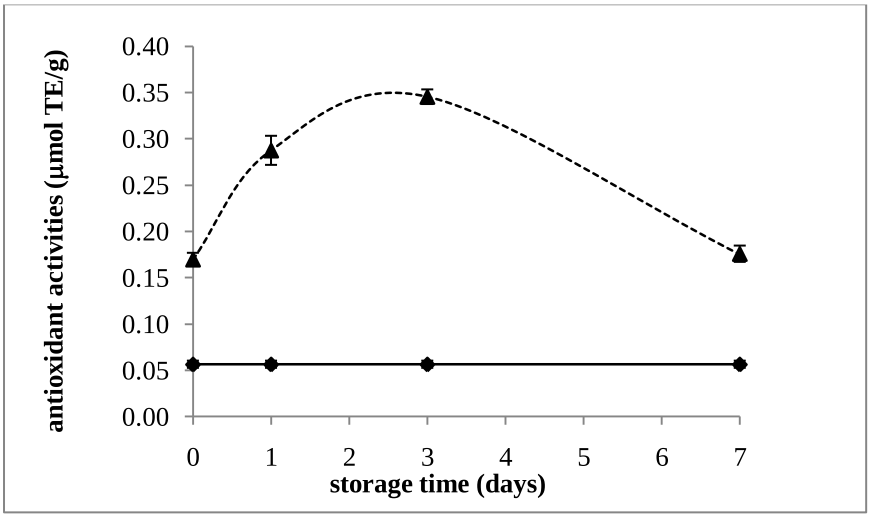


Figure 5.18 Antioxidant activities of colorant-added jelly determined by DPPH assay during storage at 4 °C for 1 week (—◆— syn; synthetic colorant-added jelly and ---▲---DF colorant-added jelly)

For ORAC assay: The results of antioxidant activities determined by ORAC assay are shown in **Figure 5.19** and **Appendix P**. The average antioxidant activities of synthetic and DF colorant-added jelly were 0.04±0.00 and 0.16±0.00 µmol TE/g, respectively at day 0 of storage. The results showed a significant difference ($p \leq 0.05$) of both samples during storage time for 1 week. The antioxidant activities of DF colorant-added jelly tended to increase after the first day until day 3 of storage with no significant differences ($p > 0.05$). After day 3 of storage, they decreased slightly with a significant difference ($p \leq 0.05$).

From the results of antioxidant activities determined by DPPH and ORAC assay, they seem to agree well with each other. They indicated a similar trend of antioxidant activities in the products during storage time for 1 week.

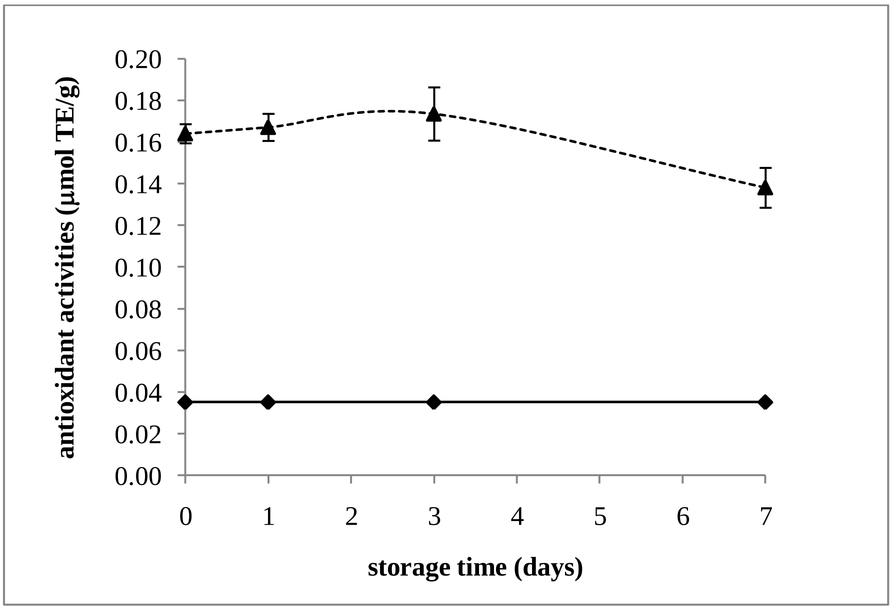


Figure 5.19 Antioxidant activities of colorant-added jelly determined by ORAC assay during storage at 4 °C for 1 week (—◆— syn; synthetic colorant-added jelly and ---▲---DF colorant-added jelly)

5.5 Sensory evaluation of food products

5.5.1 Sensory evaluation of pasteurized milk

The color appearance of 4 milk samples is illustrated in the **Figure 5.20**. The results of preference and intensity tests are shown in **Table 5.1**.

For preference test, the panelists preferred the synthetic color with a significant difference ($p \leq 0.05$) among the color scores of the 4 milk samples. Both of synthetic colorant added milk presented higher color scores than both of DF colorant added milk. The color scores of milk added synthetic colorant before and after heating were like moderately (7.60±1.07 and 7.50±1.11, respectively). On the other hand, the color scores of milk added DF colorant before and after heating were like slightly (6.60±1.50 and 6.93, respectively). Nevertheless, the color of pasteurized milk added with DF colorant after heating seemed to be comparable with the one added with the synthetic color. For the preference of odor, the scores of milk added with synthetic colorant before and after heating were like slightly (6.20±1.69 and 6.57±1.76, respectively). The odor scores of milk added DF colorant before and after heating were close to like slightly (5.87±1.63 and 5.77±1.50, respectively). However, they were not significantly different for odor preference among all milk samples ($p > 0.05$).

For intensity test, the panelists gave the highest intensity of color to milk added DF colorant after heating (8.03±2.45) and the lowest to milk added DF colorant before heating (6.72±2.70). Nevertheless, they were not significantly different among 4 milk samples ($p > 0.05$). For intensity test of odor, the panelists gave a small difference with no significant differences ($p > 0.05$) in intensity of odor which was in range of 4.48 to 6.48. The odor intensity of milk added with DF colorant before heating showed the lowest intensity (4.48±3.08) and the highest was milk added with synthetic colorant after heating (6.48±3.32).

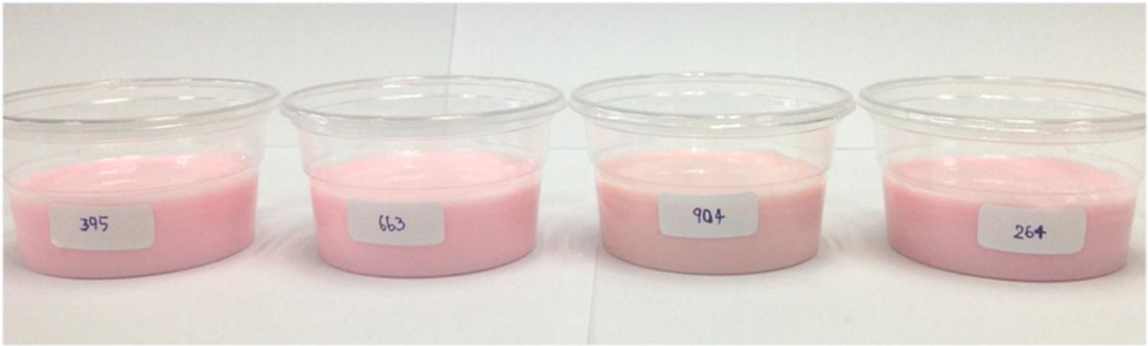


Figure 5.20 Pasteurized milk samples for sensory evaluation
(395; milk added synthetic colorant before heating, 663; milk added synthetic colorant after heating, 904 milk added DF colorant before heating,
and 264; milk added DF colorant after heating)

Table 5.1 Sensory scores of colorant-added pasteurized milk in terms of color and odor

Samples	**Preference**		**Intensity**	
	Color	**Odor**	**Color**	**Odor**
sah	7.60 ± 1.07^{a}	6.20 ± 1.69^{a}	7.93 ± 2.68^{a}	5.49 ± 2.68^{a}
sha	7.50 ± 1.11^{ab}	6.57 ± 1.76^{a}	7.52 ± 2.41^{a}	6.48 ± 3.32^{a}
dfah	6.60 ± 1.50^{c}	5.87 ± 1.63^{a}	6.72 ± 2.70^{a}	4.48 ± 3.08^{a}
dfha	6.93 ± 1.17^{bc}	5.77 ± 1.50^{a}	8.03 ± 2.45^{a}	5.01 ± 2.80^{a}

*Values within the same column with different superscripts are significantly different ($p \leq 0.05$)

5.5.2 Sensory evaluation of jelly

The color appearance of jelly samples is illustrated in the **Figure 5.21**. The results of preference and intensity tests are shown in **Table 5.2**.

For preference test, the panelists gave the mean score of 6.73±1.65 and 6.70±1.29 (like slightly) of the color preference scores for synthetic colorant and DF colorant-added jelly, respectively. For odor preference scores, the score of synthetic colorant and DF colorant added jelly were 5.70±1.36 and 5.73±1.26, respectively (neither like nor dislike). All the color and odor preference scores of both samples were not significantly different (p>0.05).

For intensity test, the color intensity score of synthetic and DF colorant-added jelly was 6.11±2.69 and 7.06±2.85, respectively with no significant differences (p>0.05). For the intensity of odor, the panelists gave a very small score for the intensity of odor to both samples. The odor intensity score of synthetic and DF colorant added jelly was 2.80±2.56 and 3.31±2.21, respectively with no significant differences (p>0.05).

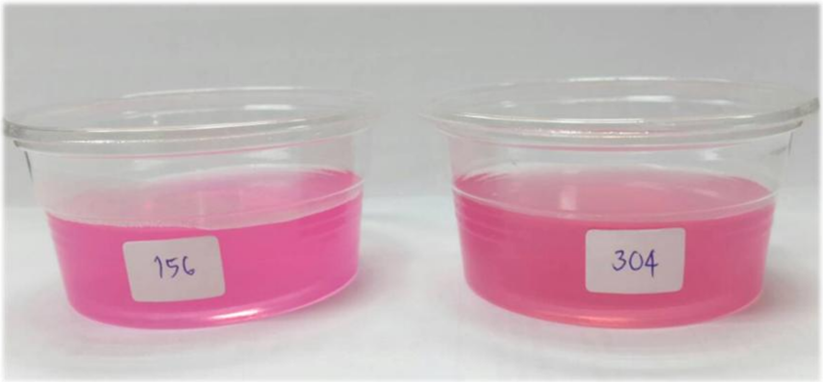


Figure 5.21 Jelly samples for sensory evaluation (156; synthetic colorant-added jelly and 304; DF colorant-added jelly)

Table 5.2 Sensory scores of colorant-added jelly in terms of color and odor

Samples	**Preference**		**Intensity**	
	Color	**Odor**	**Color**	**Odor**
Synthetic colorant	6.73±1.65	5.70±1.36	6.11±2.69	2.80±2.56
DF colorant	6.70±1.29	5.73±1.26	7.06±2.85	3.31±2.72

*Values without superscripts in the same column indicated no significant differences (p>0.05)

CHAPTER VI

DISCUSSION

6.1 Dragon fruit peel

Dragon fruit (*Hylocereus polyrhizus*) peel was obtained from different markets around the Salaya, Nakhon Pathom area. It was collected and pooled together to obtain a sizable batch of raw material for use during the study. The reason was to mimic the actual situation of raw material gathering from by-products of agricultural materials available from sources in the market or food processing facility. The peel was washed, cleaned without cutting and stored in a freezer at -20 °C. To reduce the variation of analysis, the peel was mixed well and taken in a randomized manner at the time of usage. During storage betacyanin content in the peel may decrease when compare to the fresh peel. The color of dragon fruit peel seemed to fade and became more yellow. However, a previous study reported that the loss of unclarified betacyanin was lower than the purified betacyanin (85). Therefore, the loss of betacyanin in dragon fruit peel during frozen storage in this study was expected to be minimal.

6.2 Extraction of betacyanin from dragon fruit peel

Extraction of betacyanin from dragon fruit peel by the present method yielded 1.25 mg/ml concentrated colorant or 2.72 mg/100 g fresh dragon fruit peel. The estimated cost was around 35 baht/100 g fresh dragon fruit peel which was calculated from the cost of 95% ethanol, electricity charge, deionized water, and miscellaneous expenses. The cost of ethanol, however, could be less since it could be reused in 2 more extraction although the yield may decline in subsequent extraction.

After ethanolic extraction, the betacyanin containing extract was dissolved in deionized water to make betacyanin-rich aqueous solution (DF colorant). Dissolving betacyanin in deionized water could avoid the degradation of betacyanin when compared to dissolving in alcohol (86). The concentrated DF colorant showed a dark red color whereas the diluted colorant exhibited a bright red-purple color.

Betacyanin was extracted from dragon fruit peel to produce DF colorant by using 95% ethanol at room temperature. In the previous studies, they mostly used distilled water for extraction of betacyanin from dragon fruit pulp and peel because betacyanin is a water soluble pigment. Distilled water could release betacyanin from the peel better than ethanol. Moreover, they reported that extraction at 100 °C is the best temperature. High temperature destroyed the tissue of the peel and increased the efficiency of betacyanin extraction. However, the color of betacyanin changed from red-purple to scarlet red because of the temperature (57, 58). In the present study, 95% ethanol and extraction at room temperature were chosen because using distilled water as an extraction solvent also released the water soluble fiber from dragon fruit peel resulting in the solvent became like mucilage. The step of filtration and evaporation then could not be continued. The water soluble fiber was precipitated by ethanol, so using ethanol could solve this problem. Furthermore it helped to shorten the preparation time and did not require a long time and high temperature for evaporation (60, 61). This could provide an advantage in preventing the degradation and color change of betacyanin during evaporation. Furthermore, the waste of ethanol after evaporation could be reused as the extraction solvent for 2-3 times. After reusing it for 2-3 times, the percentage of ethanol was too low because of contamination of water from the peel. Then the resulting extraction solution once again became mucilage.

6.3 Stability of betacyanin in dragon fruit peel colorant

To study the stability of DF colorant, DF colorant was kept in the amber glasses bottles at 4 °C for 8 weeks.

6.3.1 Color parameters

The change in color parameters was obvious after one week of storage. The a* value (redness) tended to decrease whereas the b* value (yellowness) tended to increase. Betacyanin is sensitive to light and storage temperature. The appearance of yellowness might be because of the presence of betaxanthin which represented yellow-orange color or the degradation of betacyanin. The degradation of betacyanin during storage time caused the loss of red color. Betacyanin degraded into cyclodopa-5-O-glycoside and betalamic acid which represent colorless and yellow color, respectively. Due to this mechanism, it led to loss of red color and increased appearance of yellow color (7, 87).

6.3.2 Betacyanin content

In term of betacyanin content, the average betacyanin content at day 0 of storage was 1.25 mg/ml or 2.72 mg/100 g fresh dragon fruit peel which was similar to the study of Harivaindaran *et al*. (58). Otherwise, the betacyanin content was less than the result reported by Jamilah *et al*. (4). In that study, freeze-dried dragon fruit peel was blended and diluted with buffer solution. This may be due to the difference in the variety of dragon fruit used in the two studies as well as the growing, harvesting and storage condition. In addition, betacyanin content extracted from dragon fruit peel showed a lower value than certain plants such as red beetroot (84.26 mg/100g fresh fruit) and *Opuntia* fruit (14.3 mg/100g fresh fruit) (88, 89).

For the stability of DF colorant during storage, betacyanin content decreased after storage for 1 week. According to the study of Harivaindaran *et al.*, their results showed that resuspended mixture of dried pigment from dragon fruit peel was stable up to 7 days (58). Moreover, the results of betacyanin content corresponded to the color parameters as mentioned earlier. Both the red color and betacyanin content tended to decrease at day 7 of storage. In general, betacyanin is the major pigment in DF colorant. Thus the decrease of betacyanin content during storage time caused the change of color appearance. It was observed that betacyanin is a rather unstable pigment. Hence, in the previous studies, they reported that adding flavonoids or ascorbic acid into betacyanin solution could help to prevent the degradation of betacyanin (86, 90, 91).

6.3.3 Antioxidant activities

Antioxidant activities of DF colorant determined by DPPH and ORAC assay were 19.37 and 222.18 μmol TE/ml (42.25 and 485.06 μmol TE/100 g), respectively. The results from different antioxidant activity assays could be explained by the difference in the mechanism of the methods in measuring the antioxidant activity. Nevertheless, the ORAC assay has been suggested to provide greater sensitivity (75). In term of the concentrated sample, the antioxidant activities were higher than the values from other plants in the cactus pear family (92). In term of fresh weight, the values were lower than that given in the previous study of Gian *et al*. The antioxidant activity of dragon fruit peel determined by DPPH assay was 195 µmol TE/ml (93). However, the antioxidant activities of the present study obtained from betacyanin concentrate whereas it was analyzed from the whole peel in the previous study. On the other hand, the antioxidant activities of DF colorant were in a similar proximate range to the antioxidant activities of some fruits such as apple and papaya as reported by Carlsen *et al*. (94). **Table 6.1** shows the antioxidant activities of some fruits and vegetables.

After 2 months of storage, the antioxidant activities slightly decreased as analyzed by both of the determination methods. These results were agreeable with the results of betacyanin content as mentioned in section 5.2.2. Betacyanin is the major antioxidant material in DF colorant (95). Then the antioxidant activities tended to decrease when betacyanin content declined.

Table 6.1 Samples of antioxidant activities in fruits and vegetables (94)

Fruits/Vegetables	Antioxidant activities (mmol/100g)
Chilli (red/green)	2.4
Apple	0.4
Strawberry	2.1
Broccoli	0.5
Orange	0.9
Papaya	0.6
Pomegranate	1.8
Artichoke	3.5
Mango (dried)	1.7

6.4 Effect of pH on the stability of dragon fruit peel colorant

6.4.1 Color parameters

The pH value of DF colorant after extraction was 4.83. It was adjusted to pH 3, 5, and 7 for studying the effect of pH on the stability of the colorant. From the results in section 5.3.1, DF colorant at pH 7 showed relatively unstable values, while DF colorant at pH 5 was more stable which was agreeable with a previous study. They reported that the optimum pH value for the maximum stability of betacyanin was pH 5. Differences in the pH value affected on hydrolytic cleavage of aldimine bond, thus betacyanin degraded into cyclo-DOPA-glycoside and betalamic acid. It led to a loss in red color and enhanced the appearance of yellowness (89, 96). The original pH value of the dragon fruit peel seemed to be the best condition for the stability.

In case of colorant appearance, DF colorant at different pH values indicated different color values, but they were still in the same shade when visually observed. The previous studies showed that betacyanin is quite stable in a wide pH range from 3-7 (96-98). Hence, DF colorant might be applied in various kinds of food products**.**

6.4.2 Betacyanin content

Betacyanin content of DF colorant was highest in the colorant adjusted to pH 5, followed by pH 7 and 3. These were similar to the study of Reynoso *et al.* (99). They found the highest loss of pigment at a highly acidic condition, and the lowest loss was found at pH 5. Hence, variation of pH value affected on the degradation of betacyanin as mentioned in section 6.5.1. Furthermore, betacyanin content of samples seemed to increase at day 3 of storage time. It might be because of the regeneration of betacyanin after storage at 4 ºC as observed in previous studies. After degradation of betacyanin, it could be regenerated well at low temperature (5 °C) in comparison with higher temperature (20°C). The mechanism of the regeneration is presented in **Figure 6.1**. However, they observed the decreasing of its content after longer storage which accorded to the results of this present study (100, 101).

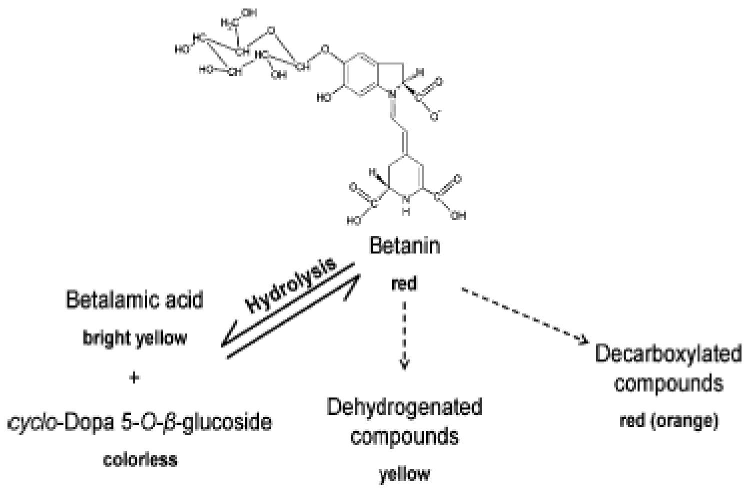


Figure 6.1 Mechanism of the degradation and regeneration of betacyanin (betacyanin $\rightleftharpoons$ cyclo-DOPA + betalamic acid) (56)

6.4.3 Antioxidant activities

The antioxidant activities of the samples at pH 7 presented the lowest values as measured by both of the determination methods. These did not relate to the results of betacyanin content as reported in section 5.3.2. It was possible that betacyanin content did not contribute to the total antioxidant activities of the colorant extract. A previous study also found the least stable antioxidant activity at pH 7 in trolox and α-tocopherol (102). According to the study of Ruenroenklin *et al.*, they found that pH 3-4 provided high antioxidant activities in litchi fruit pericarp tissue (103). During storage time, the values of DPPH assays seemed to decrease less than ORAC assay. As mentioned earlier, determination by ORAC assay seem to entail higher sensitivity.

6.5 Application of dragon fruit peel colorant in food products

DF colorant was applied in two different types of food products. For sensory evaluation, each sample was served to the panelists for evaluation of preference and intensity in term of color and odor. The products were also determined for the stability by measurement of color and antioxidant activities, and sensory evaluation.

6.5.1 Sensory evaluation of milk

Four different milk samples were served to the panelists. In term of color preference, the panelists gave a significantly different score for milk added with DF colorant before heating (dfah) ($p \leq 0.05$). The results agreed with the results in **Figure 5.20** which showed that the difference of color could be observed visually. The color of dfah was more clouded than the others. In term of color intensity, the lowest intensity was also given to the sample dfha. However, it was not significantly different ($p > 0.05$). For the results of odor, all results indicated no significant differences. The panelists did not detect any unusual smell in the DF colorant-added milk. Different preparations of milk showed that DF colorant was not stable with thermal treatment. To avoid the degradation of betacyanin of DF colorant with thermal treatment products, adding colorant after thermal treatment should be the way to apply this kind of colorant. In general, adding the food additives after thermal treatment is performed as part of the commercial pasteurization (54, 104).

6.5.2 Sensory evaluation of jelly

Two samples of jelly which were added with synthetic and DF colorant were served to the panelists. This study did not attempt to study the effect of thermal treatment because in the preparation of jelly the colorant is usually added after heating the fully dissolved jelly powder. Thus this study showed the results of color and odor difference affected by the different kinds of colorant. **Figure 5.21** presents different color between 2 cups of jelly. Synthetic colorant-added jelly gave brighter pink color which could be observed visually. Some panelists commented that synthetic colorant-added jelly looked unnatural. They could detect the different kinds of colorant. Nevertheless, the panelists gave no significant difference in the evaluation scores

($p>0.05$). This meant DF colorant could well be used instead of a synthetic colorant in this type of product.

When considering both of the model products, the results of sensory evaluation showed DF colorant could be used as an alternative to synthetic colorants. The color and odor of food added with DF colorant were accepted by the panelists. Furthermore, the panelists could not detect any unusual odor in the DF colorant added products. In pasteurized milk, the color of the two colorant-added milk products was so similar although DF colorant would preferably be added after heat treatment. The difference of appearance of colorant-added jelly with different type of color (i.e. DF and synthetic colorant) was detected easily. However, some panelists preferred the DF colorant-added jelly because the synthetic colorant-added jelly looked unnatural.

6.6 Stability of DF colorant in food products

The stability studies of DF colorant presented in section 5.2 showed that it was unstable during storage for a long time. Besides, pH value of the products could also affect its stability. The pH value of pasteurized milk and jelly was 6.5 and 5.8, respectively. Therefore, short shelf-life and suitable pH products e.g. pasteurized food, were chosen for the application test in this study.

6.6.1 Pasteurized milk

For color parameters: From all results of color parameters, there was an effect of heating on the stability of the colorant. DF colorant was not stable during heat treatment which could be observed from the significant difference of a* and b* values of the products added with DF colorant before compared to after heat treatment. Heating leads to degradation of betacyanin into cyclo-DOPA and betalamic acid (85, 100). The results showed decreasing redness and increasing yellowness due to both heating and storage time. These results were in accordance with the results of stability study of betacyanin in section 5.2.1. According to **Figure 5.13**, the color of DF colorant-added pasteurized milk seemed to fade slightly even so it was not easy to detect by visual observation.

For antioxidant activities: The antioxidant activities were higher in both milk samples added with DF colorant before and after heating when compared to milk added with synthetic colorant and normal dairy products (approximately 1.4 μmol TE/ml) (94). Furthermore, the effect of heating on antioxidant activities was studies in this part. The results showed that adding DF colorant before pasteurization resulted in lower antioxidant activities as determined by both of the analytical methods (DPPH and ORAC). Betacyanin content tended to decline with thermal treatment at 70 °C (105). Then it was expected that a decrease in antioxidant activities would be observed. Interestingly, the values increased after one day of storage which indicated by both DPPH and ORAC assays. These might be because of the regeneration of betacyanin after heating and storage at low temperature as explained in section 6.4.2.

6.6.2 Jelly

For color parameters: When compared the color parameters between both of jelly, the color parameters of synthetic and DF colorant-added jelly were notably different. Nevertheless, the sensory evaluation results showed that they were accepted by the panelists. During storage, the results in section 5.4.2 showed a slight change of color parameters but the color could be noticed as faded by visual observation (**Figure 5.17**). The reason was the degradation of betacyanin during storage time as mentioned earlier. The present study confirmed that betacyanin is an unstable pigment even it was not processed by high temperature. This may be due to the antioxidant activities of the pigment which make it sensitive to exposure to light and air (oxygen).

For antioxidant activities: The antioxidant activities of DF colorant-added jelly exhibited much higher values (around 4 times) when compared to synthetic colorant-added jelly. Nevertheless, these antioxidant activities were still low when compared to other food product such as desserts and cakes (approximately 4.5 μmol/g) (94). The reasons might be the based ingredient which was agar powder has very low antioxidant activities. Furthermore, the colorant was added in a small amount to obtain the suitable color of jelly. It was, however, interesting to note that an increase in antioxidant activities was observed as measured by both of the determination methods during storage time. It was similar to the results of antioxidant activities in milk as reported in section 5.4.1.2 and 6.6.1.

CHAPTER VII
CONCLUSION

Dragon fruit peel, red flesh variety, could be extracted with 95% ethanol at a ratio of 1:2 (w/v) and evaporated to obtain betacyanin-rich concentrate (DF colorant). The concentrate DF colorant exhibited a dark red, and the diluted DF colorant exhibited a bright red-purple color. This color shade of DF colorant was similar to erythrosine synthetic colorant which is widely used in the food processing and food service industry.

The present study revealed that DF colorant was a rather unstable pigment. The stability was affected by pH, heat, and storage time. Its betacyanin content and antioxidant activities showed a decreasing trend during 2 months of storage. For the pH study, the color of DF colorant adjusted to different pH values appeared to be the same shade. Whereas, the colorant adjusted to pH 7 presented a bit faded color when compared to the colorant adjusted to pH 3 and 5. The colorant adjusted to pH 5 provided the best values in term of color parameters, betacyanin content, and antioxidant activity. Thus it seemed to be the best condition while the colorant at pH 7 was the least stable.

The effect of heating on the stability of DF colorant was studied by pasteurization of colorant-added milk at 72 °C for 15 s. Heat treatment caused a decrease in the red color. The degradation of betacyanin led to the loss of red color and the colorant became more yellow. Furthermore, a decrease in antioxidant activity was found in pasteurized milk with DF colorant added after heating. Nevertheless, betacyanin could regenerate during storage at a low temperature (4^{o}C) for 1-3 days.

For the application of DF colorant in food products, both pasteurized milk and jelly were accepted by 30 untrained panelists in terms of both color and odor. Although food products added with a synthetic colorant exhibited a brighter color, they were criticized as unnatural looking. Hence, DF colorant could be considered as an alternative colorant for use instead of synthetic colorants. There was no unusual odor contributed by DF colorant in the tested food products. DF colorant can be prepared for use in various types of foods, even in solid or liquid form, or at different pH values. Nevertheless, since DF colorant was not stable with heat treatment, high temperature process should be avoided. It would be suitable to add the colorant after heating the product such as in milk, jelly, ice cream, or beverage. DF colorant could also contribute antioxidant activities to foods even though the value may be small due to a relatively low level of usage.

In conclusion, dragon fruit peel can be used as a source of natural food colorant with functional properties. It could provide a means to increase the value of agricultural by-products. Further research should be carried out on the stabilization study of betacyanin as affected by heat and storage time to obtain better utilization of the DF colorant. The possible studies could include: 1) adding flavonoids or ascorbic acid into betacyanin solution which could help prevent the degradation of betacyanin as described in section 6.3.2 or 2) extracting and preparing betacyanin in a powder form by spray-drying to improve the storage ability. Betacyanin in a powder form has a lower water activity (a_w) value than that in an aqueous form. A previous report showed that betacyanin with lower a_w exhibited good stability (2). Moreover, the safety of dragon fruit peel should be confirmed by checking for contamination of pesticides, mold killers or chemical fertilizers which may be used during the cultivation.

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APPENDICES

APPENDIX A

DETERMINATION OF ANTIOXIDANT ACITIVITIES BY DPPH FREE RADICAL SCAVENGING ASSAY

Reagents:

- 2,2-diphenyl-1-picrythydrazyl (DPPH)
- 95% Ethanol
- Trolox ((±)-6-hydroxyl-2,5,7,8-tetramethylchomane-2-carboxylic acid, 97%), Sigma Aldrich # 238813

Equipment:

- Analytical balance (Metter Toledo, AG 204)
- Vortex
- UV-visible spectrophotometer (UV-1601, Shimadzu, Japan)

Reagent preparation:

1. 0.15 mM DPPH reagent

0.006 g of DPPH was dissolved with 95% ethanol and adjusted to 100 ml in a 100-ml volumetric flask. DPPH reagent was kept in an opaque bottle to protect from the light. The reagent was prepared freshly before the determination.

2. 8 mM Trolox standard solution

0.1001 g of trolox was precisely weighed. It was dissolved with 95% ethanol and adjusted volume to the mark in a 50-ml volumetric flask. The solution was made serial solution to 0.1, 0.08, 0.06, 0.04, 0.02, and 0.01 mM for the analysis.

APPENDIX B
DETERMINATION OF ANTIOXIDANT ACITIVITIES BY OXYGEN RADICAL ABSORBANCE CAPACITY (ORAC ASSAY)

Reagents:

- Dipotassium hydrogen phosphate (K_2HPO_4), pa, Merck
- Potassium dihydrogen phosphate (KH_2PO_4), pa, Merck
- 2,2’-azobis (2-amidinopropane) dihydrochloride (AAPH), Sigma Aldrich # 440914
- Fluorescein sodium salt, Sigma Aldrich # 166308
- Trolox ((±)-6-hydroxyl-2,5,7,8-tetramethylchomane-2-carboxylic acid, 97%), Sigma Aldrich # 238813

Equipment:

- Luminescence spectrometer (LS55, Perkin Elmer, USA)
- Vortex

Reagent preparation:

1. **ORAC buffer stock solution**

102.07 g of KH_2PO_4 was dissolved with deionized water and adjusted volume to the mark in a 1000-ml volumetric flask. It was yield 1000 ml of 0.75 M of KH_2PO_4 stock solution (stored at 4 °C). To prepare 0.75 M of K_2HPO_4 stock solution, 130.64 g of K_2HPO_4 was dissolved with deioxized water and filled up the volume to the mark in a 1000-ml volumetric flask (stored at 4 °C). After that, 603 ml of K_2HPO_4 stock solution was mixed with 351 ml of KH_2PO_4 stock solution to yield the ORAC buffer stock solution.

2. **ORAC buffer working solution**

Pipette 100 ml of ORAC buffer stock solution into a 1000-ml volumetric flask and filled up the volume nearly to the mark with deionized water. Adjusted pH to

7.2 with NaoH concentrated solution then filled up the volume with deionized water again. ORAC buffer working solution was kept at 4 °C.

3. 153 mM APPH

Weigh 0.414 g of AAPH in to a 10-ml volumetric flask. The volume was filled up with ORAC buffer working solution. The solution was prepared freshly and kept in the ice bath before using.

4. Fluorescein concentrated solution

0.045 g of fluorescein was diluted with 100 ml of ORAC buffer working solution in a 100-ml volumetric flask. It yielded 100 ml of 8.37×10^{-4} mM fluorescein concentrated solution. It was stored in an amber bottle at 0 °C.

5. Fluorescein stock solution

Pipette 100 μl of fluorescein concentrated solution and added with 20 ml of ORAC buffer working solution. Fluorescein stock solution was kept in an amber bottle at 4 °C.

6. Fluorescein working solution

Pipette 1.95 ml of fluorescein stock solution into a 100-ml volumetric flask and adjusted the volume with ORAC buffer working solution.

7. 1000 μM Trolox stock solution

0.025 g of trolox was dissolved with ORAC buffer working solution and adjusted the volume to the mark in a 100-ml volumetric flask. 1000 μM trolox stock solution was made serial dilution to 100, 50, 25, 12.5, and 6.25 μM for the analysis.

APPENDIX C

L* a* b* color parameter of DF colorant during storage time for 2 months

Storage time (days)	L*	a*	b*
0	45.49±0.41^{d}	20.88±0.19^{b}	-0.62±0.19^{f}
3	45.55±0.12^{d}	21.42±0.09^{a}	-0.54±0.05^{f}
7	45.75±0.08^{d}	20.41±0.19^{b}	0.26±0.02^{e}
14	45.89±0.53^{d}	17.25±0.66^{c}	0.99±0.35^{d}
28	49.55±0.31^{c}	12.83±0.10^{d}	2.55±0.02^{c}
42	50.60±0.38^{b}	9.74±0.06^{e}	3.85±0.11^{b}
56	51.70±0.20^{a}	7.95±0.03^{f}	4.54±0.06^{a}

[1] Values in a column with different superscript are significantly different ($p \leq 0.05$). One way ANOVA followed by the Duncan's Multiple Range Test were used.

APPENDIX D

Betacyanin content of DF colorant during storage time for 2 months

Storage time (days)	Betacyanin content (mg/ml)
0	1.25±0.01^{a}
3	1.25±0.01^{a}
7	1.22±0.01^{b}
14	1.00±0.00^{c}
28	0.87±0.00^{d}
42	0.73±0.00^{e}
56	0.61±0.00^{f}

[1] Values in a column with different superscript are significantly different ($p \leq 0.05$). One way ANOVA followed by the Duncan's Multiple Range Test were used.

APPENDIX E

Antioxidant activities of DF colorant determined by DPPH assay during storage time for 2 months

Storage time (days)	**Antioxidant activities (μmol TE/ml)**
0	19.37±0.08^{a}
3	17.00±0.26^{b}
7	15.28±0.32^{c}
14	15.13±0.29^{c}
28	14.68±1.04^{c}
42	13.05±0.49^{d}
56	12.77±0.25^{d}

[1] Values in a column with different superscript are significantly different ($p \leq 0.05$). One way ANOVA followed by the Duncan's Multiple Range Test were used.

APPENDIX F

Antioxidant activities of DF colorant determined by ORAC assay during storage time for 2 months

Storage time (days)	Antioxidant activities (μmol TE/ml)
0	222.18±25.85^{ab}
3	223.92±8.99^{a}
7	193.98±27.11^{bc}
14	189.31±10.15^{c}
28	186.34±11.56^{c}
42	156.80±7.49^{d}
56	147.72±8.14^{d}

[1] Values in a column with different superscript are significantly different ($p \leq 0.05$). One way ANOVA followed by the Duncan's Multiple Range Test were used.

APPENDIX G

L* a* b* color parameter of DF colorant at different pH values during storage time for 15 days

CIE color parameters	Storage time (days)	Storage condition		
		pH 3	pH 5	pH 7
L*	0	49.26±0.10^{cB}	46.62±0.26^{bC}	52.92±0.35^{aA}
	3	48.85±0.08^{dA}	46.45±0.15^{bB}	48.97±0.29^{dA}
	6	49.35±0.06^{cA}	46.04±0.11^{cC}	48.37±0.07^{eB}
	9	50.37±0.10^{bB}	47.20±0.11^{aC}	50.69±0.06^{bA}
	12	50.35±0.05^{bA}	47.33±0.07^{aC}	49.79±0.01^{cB}
	15	50.64±0.01^{aA}	47.14±0.02^{aC}	49.27±0.01^{dB}
a*	0	18.67±0.03^{bB}	23.55±0.06^{aA}	15.08±0.14^{dC}
	3	18.82±0.05^{aB}	23.64±0.05^{aA}	15.23±0.16^{dC}
	6	18.56±0.07^{cB}	23.07±0.13^{bA}	17.84±0.03^{bC}
	9	16.27±0.06^{dB}	22.92±0.08b^{cA}	12.56±0.04^{eC}
	12	16.27±0.01^{dC}	23.00±0.09^{bB}	16.38±0.02^{cA}
	15	16.23±0.03^{dC}	22.79±0.04^{cA}	18.43±0.02^{aB}
b*	0	-5.12±0.08^{cB}	-6.54±0.04^{bC}	-3.29±0.06^{bA}
	3	-5.15±0.03^{cB}	-6.72±0.04^{aC}	-3.43±0.05^{cA}
	6	-5.17±0.05^{cA}	-6.27±0.07^{cC}	-5.51±0.03^{fB}
	9	-4.47±0.07^{aB}	-6.66±0.03^{cC}	-3.02±0.00^{aA}
	12	-4.52±0.02^{aB}	-6.73±0.06^{cC}	-4.13±0.01^{dA}
	15	-4.79±0.01^{bA}	-6.69±0.01^{cC}	-4.96±0.01^{eB}

[1] Values in a column with different superscript (a, b) are significantly different ($p \leq 0.05$). One way ANOVA followed by the Duncan's Multiple Range Test were used.

[2] Values in a row with different superscript (A, B) are significantly different ($p \leq 0.05$). One way ANOVA followed by the Duncan's Multiple Range Test were used.

APPENDIX H

Betacyanin content of DF colorant at different pH value during storage time for 15 days

Storage time (days)	Betacyanin content (mg/ml)		
	pH 3	pH 5	pH 7
0	1.34±0.00^{cC}	2.27±0.01^{bA}	1.63±0.01^{eB}
3	1.70±0.01^{aC}	2.29±0.01^{aA}	1.88±0.00^{aB}
6	1.57±0.00^{bC}	2.19±0.01^{cB}	1.78±0.02^{cA}
9	1.33±0.02^{cC}	2.18±0.01^{cA}	1.81±0.01^{bB}
12	1.26±0.01^{dC}	2.08±0.00^{dA}	1.69±0.00^{dB}
15	1.24±0.00^{eC}	2.10±0.03^{dA}	1.61±0.01^{fB}

[1] Values in a column with different superscript (a, b) are significantly different ($p \leq 0.05$). One way ANOVA followed by the Duncan's Multiple Range Test were used.

[2] Values in a row with different superscript (A, B) are significantly different ($p \leq 0.05$). One way ANOVA followed by the Duncan's Multiple Range Test were used.

APPENDIX I

Antioxidant activities of DF colorant at different pH value determined by DPPH assay during storage time for 15 days

Storage time (days)	Antioxidant activities (µmol TE/ml)		
	pH 3	**pH 5**	**pH 7**
0	23.24 ± 0.09^{aA}	22.88 ± 0.41^{aA}	8.24 ± 0.55^{aB}
3	21.56 ± 0.41^{bA}	21.76 ± 0.16^{bA}	6.15 ± 0.17^{bB}
6	19.69 ± 0.72^{cA}	20.01 ± 0.12^{dA}	5.58 ± 0.05^{cB}
9	18.06 ± 0.12^{eB}	19.27 ± 0.07^{eA}	5.60 ± 0.04^{cC}
12	$18.44\pm0.22d^{eB}$	19.58 ± 0.17^{eA}	$5.17\pm0.11c^{dC}$
15	19.00 ± 0.14^{dB}	20.54 ± 0.06^{cA}	5.02 ± 0.05^{dC}

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[1] Values in a column with different superscript (a, b) are significantly different ($p\leq0.05$). One way ANOVA followed by the Duncan's Multiple Range Test were used.

[2] Values in a row with different superscript (A, B) are significantly different ($p\leq0.05$). One way ANOVA followed by the Duncan's Multiple Range Test were used.

APPENDIX J

Antioxidant activities of DF colorant at different pH value determined by ORAC assay during storage time for 15 days

Storage time (days)	Antioxidant activities (µmol TE/ml)		
	pH3	pH5	pH7
0	180.20 ± 10.78^{aB}	203.49 ± 12.75^{aA}	154.87 ± 10.70^{aC}
3	153.64 ± 10.39^{bA}	185.90 ± 11.33^{bA}	164.46 ± 22.47^{aA}
6	174.53 ± 19.16^{abA}	178.12 ± 7.54^{bA}	168.89 ± 10.57^{aA}
9	160.15 ± 14.87^{abA}	173.83 ± 3.69^{bA}	132.30 ± 2.70^{bB}
12	150.08 ± 7.50^{bB}	184.70 ± 6.29^{bA}	69.95 ± 9.27^{cC}
15	58.91 ± 10.66^{cA}	53.01 ± 7.39^{cAB}	38.77 ± 6.81^{dA}

[1] Values in a column with different superscript (a, b) are significantly different ($p \leq 0.05$). One way ANOVA followed by the Duncan’s Multiple Range Test were used.

[2] Values in a row with different superscript (A, B) are significantly different ($p \leq 0.05$). One way ANOVA followed by the Duncan’s Multiple Range Test were used.

APPENDIX K

L* a* b* color parameter of colorant-added pasteurized milk during storage time for 7 days

CIE color parameters	Storage time (days)	Type of milk			
		sah	sha	dfah	dfha
L*	0	69.97 ± 0.01^{aB}	70.23 ± 0.01^{aA}	68.08 ± 0.03^{dC}	67.39 ± 0.06^{cD}
	1	69.97 ± 0.01^{aB}	70.23 ± 0.01^{aA}	68.17 ± 0.01^{cC}	67.42 ± 0.04^{cD}
	3	69.97 ± 0.01^{aB}	70.23 ± 0.01^{aA}	68.73 ± 0.01^{bC}	68.22 ± 0.00^{bD}
	7	69.97 ± 0.01^{aB}	70.23 ± 0.01^{aA}	68.67 ± 0.02^{aC}	69.01 ± 0.01^{aD}
a*	0	12.46 ± 0.00^{aA}	11.81 ± 0.03^{aC}	10.79 ± 0.01^{aD}	11.89 ± 0.01^{aB}
	1	12.46 ± 0.00^{aA}	11.81 ± 0.03^{aB}	10.63 ± 0.00^{bC}	11.77 ± 0.03^{bB}
	3	12.46 ± 0.00^{aA}	11.81 ± 0.03^{aB}	10.41 ± 0.01^{cD}	11.28 ± 0.02^{cC}
	7	12.46 ± 0.00^{aA}	11.81 ± 0.03^{aB}	8.18 ± 0.08^{dC}	8.15 ± 0.04^{dC}
b*	0	1.80 ± 0.01^{aC}	2.03 ± 0.02^{aB}	2.32 ± 0.02^{dA}	1.54 ± 0.01^{dD}
	1	1.80 ± 0.01^{aD}	2.03 ± 0.02^{aC}	2.95 ± 0.01^{cA}	2.36 ± 0.07^{cB}
	3	1.80 ± 0.01^{aD}	2.03 ± 0.02^{aC}	3.82 ± 0.03^{bA}	2.86 ± 0.02^{bB}
	7	1.80 ± 0.01^{aD}	2.03 ± 0.02^{aC}	4.57 ± 0.06^{aA}	3.53 ± 0.10^{aB}

[1] Values in a column with different superscript (a, b) are significantly different ($p \leq 0.05$). One way ANOVA followed by the Duncan's Multiple Range Test were used.

[2] Values in a row with different superscript (A, B) are significantly different ($p \leq 0.05$). One way ANOVA followed by the Duncan's Multiple Range Test were used.

[3] sah= milk added synthetic colorant before heating, sha= milk added synthetic colorant after heating, dfah= milk added DF colorant before heating, dfha= milk added DF colorant after heating

APPENDIX L

Antioxidant activities of colorant-added pasteurized milk determined by DPPH assay during storage time for 7 days

Storage time (days)	Antioxidant activities (μmol TE/ml)			
	sah	**sha**	**dfah**	**dfha**
0	0.19 ± 0.02^{aAB}	0.19 ± 0.01^{aAB}	0.17 ± 0.00^{bB}	0.21 ± 0.02^{aA}
1	0.19 ± 0.02^{aB}	0.19 ± 0.01^{aB}	0.22 ± 0.01^{aA}	0.22 ± 0.01^{aA}
3	0.19 ± 0.02^{aA}	0.19 ± 0.01^{aA}	0.18 ± 0.02^{bA}	0.20 ± 0.01^{aA}
7	0.19 ± 0.02^{aA}	0.19 ± 0.01^{aA}	0.20 ± 0.02^{aA}	0.21 ± 0.01^{aA}

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[1] Values in a column with different superscript (a, b) are significantly different ($p \leq 0.05$). One way ANOVA followed by the Duncan's Multiple Range Test were used.

[2] Values in a row with different superscript (A, B) are significantly different ($p \leq 0.05$). One way ANOVA followed by the Duncan's Multiple Range Test were used.

[3] sah= milk added synthetic colorant before heating, sha= milk added synthetic colorant after heating, dfah= milk added DF colorant before heating, dfha= milk added DF colorant after heating

APPENDIX M

Antioxidant activities of colorant-added pasteurized milk determined by ORAC assay during storage time for 7 days

Storage time (days)	Antioxidant activities (μmol TE/ml)			
	sah	**sha**	**dfah**	**dfha**
0	25.33±2.27^{aA}	24.46±3.07^{aA}	18.89±0.57^{cB}	26.27±0.77^{cA}
1	25.33±2.27^{aC}	24.46±3.07^{aC}	33.11±3.36^{aB}	43.14±0.90^{aA}
3	25.33±2.27^{aB}	24.46±3.07^{aB}	32.79±2.56^{aA}	34.21±0.61^{bA}
7	25.33±2.27^{aB}	24.46±3.07^{aB}	25.05±1.29^{bB}	33.86±0.36^{bA}

[1] Values in a column with different superscript (a, b) are significantly different ($p \leq 0.05$). One way ANOVA followed by the Duncan’s Multiple Range Test were used.

[2] Values in a row with different superscript (A, B) are significantly different ($p \leq 0.05$). One way ANOVA followed by the Duncan’s Multiple Range Test were used.

[3] sah= milk added synthetic colorant before heating, sha= milk added synthetic colorant after heating, dfah= milk added DF colorant before heating, dfha= milk added DF colorant after heating

APPENDIX N

L* a* b* color parameter of colorant-added jelly during storage time for 7 days

CIE color parameter	Storage time (days)	Type of jelly	
		syn	df
L*	0	30.85 ± 2.02^{a}	30.29 ± 1.69^{b}
	1	30.85 ± 2.02^{a}	35.42 ± 2.70^{a}
	3	30.85 ± 2.02^{a}	33.18 ± 2.63^{ab}
	7	30.85 ± 2.02^{a}	30.60 ± 1.23^{b}
a*	0	12.23 ± 1.06^{a}	9.99 ± 1.05^{a}
	1	$12.23\pm1.06^{*a}$	9.22 ± 0.66^{a}
	3	12.23 ± 1.06^{a}	10.81 ± 1.88^{a}
	7	12.23 ± 1.06^{a}	10.68 ± 0.87^{a}
b*	0	$1.38\pm0.19^{*a}$	-0.19 ± 0.13^{a}
	1	$1.38\pm0.19^{*a}$	-0.44 ± 0.48^{a}
	3	$1.38\pm0.19^{*a}$	-0.18 ± 0.29^{a}
	7	$1.38\pm0.19^{*a}$	-0.55 ± 0.77^{a}

[1] Values in a column with different superscript (a, b) are significantly different ($p \leq 0.05$). One way ANOVA followed by the Duncan's Multiple Range Test were used.

[2] syn= synthetic colorant added jelly and df= DF colorant added jelly

[*] Values in horizontal are significantly different ($p \leq 0.05$), T-test was used.

APPENDIX O

Antioxidant activities of colorant-added jelly determined by DPPH assay during storage time for 7 days

Storage time (days)	Antioxidant activities (μmol TE/ml)	
	syn	**df**
0	0.05±0.01^{*a}	0.17±0.01^{c}
1	0.05±0.01^{*a}	0.29±0.02^{b}
3	0.05±0.01^{*a}	0.35±0.01^{a}
7	0.05±0.01^{*a}	0.18±0.01^{c}

[1] Values in a column with different superscript (a, b) are significantly different ($p \leq 0.05$). One way ANOVA followed by the Duncan's Multiple Range Test were used.

[2] syn= synthetic colorant added jelly and df= DF colorant added jelly

[*] Values in horizontal are significantly different ($p \leq 0.05$), T-test was used.

APPENDIX P

Antioxidant activities of colorant-added jelly determined by ORAC assay during storage time for 7 days

Storage time (days)	Antioxidant activities (μmol TE/ml)	
	syn	df
0	0.04±0.00^{*a}	0.16±0.00^{a}
1	0.04±0.00^{*a}	0.17±0.01^{a}
3	0.04±0.00^{*a}	0.17±0.01^{a}
7	0.04±0.00^{*a}	0.14±0.01^{b}

[1] Values in a column with different superscript (a, b) are significantly different ($p \leq 0.05$). One way ANOVA followed by the Duncan's Multiple Range Test were used.

[2] syn= synthetic colorant added jelly and df= DF colorant added jelly

[*] Values in horizontal are significantly different ($p \leq 0.05$), T-test was used.

APPENDIX Q

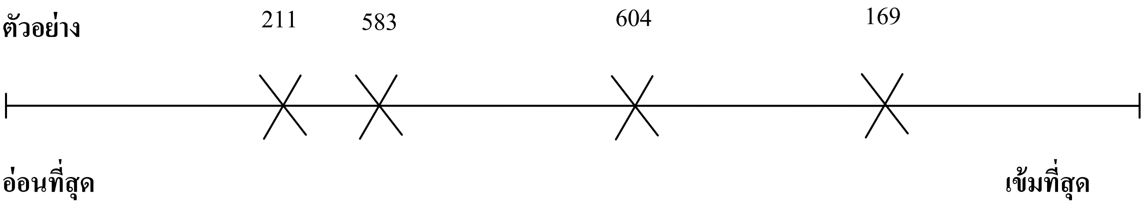
แบบประเมินความพอใจผลิตภัณฑ์แต่งสีจากธรรมชาติ

ข้อแนะนำ : โปรดประเมินตัวอย่างผลิตภัณฑ์นมปรุงแต่งจากสีธรรมชาติที่ให้ต่อไปนี้โดยการ **สังเกตสีและดมกลิ่นเท่านั้น (ไม่ต้องรับประทาน)** และตรวจสอบว่าท่านชอบ/ไม่ชอบผลิตภัณฑ์มากเพียงใด ใช้สเกลที่เหมาะสมในการแสดงทัศนคติของท่านโดยทำเครื่องหมาย ✓ ในช่องสเกลที่อธิบายความรู้สึกของท่านได้ดีที่สุด

สเกล	**สีของตัวอย่าง**				**กลิ่นของตัวอย่าง**			
	395	**663**	**904**	**264**	**395**	**663**	**904**	**264**
ชอบมากที่สุด								
ชอบมาก								
ชอบปานกลาง								
ชอบเล็กน้อย								
เฉยๆ								
ไม่ชอบเล็กน้อย								
ไม่ชอบปานกลาง								
ไม่ชอบมาก								
ไม่ชอบมากที่สุด								

ท่านได้รับตัวอย่างผลิตภัณฑ์ 4 ตัวอย่างเพื่อทดสอบความแตกต่างของความเข้มของสีและกลิ่น กรุณาทำเครื่องหมาย x บนเส้นตรง ณ ตำแหน่งที่สามารถอธิบายลักษณะตัวอย่างในความรู้สึกของท่านได้ดีที่สุด โดยให้ระบุหมายเลขของตัวอย่างไว้ยังตำแหน่งที่ท่านทำเครื่องหมาย ดังตัวอย่างที่แสดงข้างล่าง

ตัวอย่าง



ความเข้มของสี

อ่อนที่สุด เข้มที่สุด

ความเข้มของกลิ่น

อ่อนที่สุด เข้มที่สุด

ข้อเสนอแนะ

____

APPENDIX R

แบบประเมินความพอใจผลิตภัณฑ์แต่งสีจากธรรมชาติ

ข้อแนะนำ : โปรดประเมินตัวอย่างผลิตภัณฑ์วุ้นปรุงแต่งจากสีธรรมชาติที่ให้ต่อไปนี้โดยการ **สังเกตสีและดมกลิ่นเท่านั้น (ไม่ต้องรับประทาน)** และตรวจสอบว่าท่านชอบ/ไม่ชอบผลิตภัณฑ์มากเพียงใด ใช้สเกลที่เหมาะสมในการแสดงทัศนคติของท่านโดยทำเครื่องหมาย ✓ ในช่องสเกลที่อธิบายความรู้สึกของท่านได้ดีที่สุด

สเกล	**สีของตัวอย่าง**		**กลิ่นของตัวอย่าง**	
	156	**304**	**156**	**304**
ชอบมากที่สุด				
ชอบมาก				
ชอบปานกลาง				
ชอบเล็กน้อย				
เฉยๆ				
ไม่ชอบเล็กน้อย				
ไม่ชอบปานกลาง				
ไม่ชอบมาก				
ไม่ชอบมากที่สุด				

ท่านได้รับตัวอย่างผลิตภัณฑ์ 2 ตัวอย่างเพื่อทดสอบความแตกต่างของความเข้มของสีและกลิ่น กรุณาทำเครื่องหมาย x บนเส้นตรง ณ ตำแหน่งที่สามารถอธิบายลักษณะตัวอย่างในความรู้สึกของท่านได้ดีที่สุด โดยให้ระบุหมายเลขของตัวอย่างไว้ยังตำแหน่งที่ท่านทำเครื่องหมาย ดังตัวอย่างที่แสดงข้างล่าง

ตัวอย่าง

อ่อนที่สุด **เข้มที่สุด**

ความเข้มของสี

อ่อนที่สุด เข้มที่สุด

ความเข้มของกลิ่น

อ่อนที่สุด เข้มที่สุด

ข้อเสนอแนะ

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