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RESEARCH ARTICLE

Nutrition composition and anthocyanin content of mulberry fruits (*Morus alba* L.) by different ripening stage grown in the south of Thailand, applicants in sorbet and sherbet ice-cream and analysis of physical, chemical and sensory evaluation

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ABSTRACT

This experiment was study on properties of each ripening stage of mulberry fruits and conducted study of physical and chemical properties of mulberry fruit at each ripening stage and apply mulberry fruit in ice cream products. Mulberry at fully-ripened stage showed higher phenolic, flavonoid and anthocyanin content. Mulberry fruits were used to make four different types of ice cream; sorbet, sherbet, milk, and yogurt. The sensory qualities of mulberry ice cream were studied (9-point hedonic scale), and the results revealed that mulberry sherbet ice cream had the highest overall acceptability, followed by mulberry sorbet ice cream. The physical, chemical, nutrition composition, and microbiological properties of mulberry sherbet and sorbet ice cream were investigated further. Due to the presence of fresh milk in the composition, sherbet ice cream has a higher overrun and lightness (L*) than sorbet ice cream. The mulberry sherbet ice cream had more incredible energy, energy from fat, total fat, saturated fat, cholesterol, protein, total carbohydrates, calcium, dietary fiber and phenolic compounds. This study shows that mulberry has beneficial properties in terms of consumer acceptance and health benefits, particularly in mulberry sherbet ice cream. All mulberry ice creams had been sanitized and were suitable to consume. As a result, mulberry ice cream formulations can add value to agricultural products and suggested for commercialization.

Key words: Anthocyanin; Ice cream; Mulberry fruits; Ripening stage; Sherbet; Sorbet

INTRODUCTION

Mulberry fruit is categorized in the *Moraceae* family (Ashtiani, Javanmardi, Jahanbanifard, Martynenko, & Verbeek, 2021). Mulberry trees can grow throughout Thailand "Chiang Mai cultivar" is the most common cultivar for consumption (Suttisansanee et al., 2020; Suriyaprom, Kaewkod, Promputtha, Desvaux, & Tragoolpua, 2021). It has a high yield, requires about one year of cultivation to begin generating fruit and mature mulberry trees can harvest mulberry fruit for three years. Mulberry is multiple fruit with many small round shapes. Raw mulberry fruits are green with a sour flavor, while the ripe mulberry ranges from purple-red to black color from anthocyanin pigment with a unique sweet flavor (Ma et al., 2020). The maturity stage affects the quality, flavor and nutrition content of mulberry (Nayab et al., 2020). The mulberry at fully ripened stage has sweeter flavor due to five times higher free sugars content compared to semi-ripened stage (Lee & Hwang, 2017). Mulberry includes multiple nutrition elements, including fatty acids, amino acids, vitamins, minerals and bioactive compounds, such as anthocyanins, rutin, quercetin, chlorogenic acid and polysaccharides (Yuan & Zhao, 2017) Semi-ripened to the fully ripened

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stage of mulberry provided potential health benefits such as anti-atherosclerosis, anti-tumor, anti-hyperglycemin and rich in antioxidant activity, influential in anti-aging properties. Previous studies have reported Chiang Mai mulberry fruit in Thailand has contained high amounts of cyanidin, keracyanin and kuromanin as anthocyanidin and anthocyanins and it could be a protection against Alzheimer's disease (Suttisansanee et al. 2020). However, the main limitation of fully ripened mulberry has a high moisture content (84.4 to 89.7%) (Dimitrijevi, Arsić & Kostić, 2021) and providing a soft texture, making them not suitable for storage as fresh fruit. As a consequence, it is widely processed into products such as freeze-dried mulberry, mulberry concentrate juice, mulberry jam, mulberry pulp chili pastes and mulberry wine (Gahukar, 2016). Since there is not much diversity in Thailand's conventional mulberry products, mulberry ice cream might enhance mulberry supply and consumption, which would boost Thai mulberry farmers' income.

Ice cream is a frozen dairy dessert comprised of air, milk, sweeteners, water and flavoring material (Arbuckle, 2013). It may contain functional ingredients such as egg, egg products, stabilizer and emulsifier then aerated to get a fluffy and airy texture. Ice cream and ice cream-related products are categorized in frozen confectionery (Report, 2022). Ice cream origin was supposed to originate from Chinese eating culture, consuming ice dessert by blinding snow and fruit or fruit juice (Quinzio, 2009). This culture was transported to Europe in the late 13 A.D., once it was modified and developed into the current ice cream product (Arbuckle, 2013). The global ice cream market value is relatively large and continues to grow, particularly in the Asia-pacific region. In 2021, the Asia-Pacific region supplied the most to the worldwide industry with a profit share of over 42.0%. The region has experienced increased on demand, particularly from young customers (Report, 2022). However, the limitations of health concerns are considered because ice cream has a high sugar and fat content, dairy-based ice cream is not suitable for lactose-sensitive consumers (Nachay, 2019). Cow's milk allergy can be caused by IgE-mediated antibodies, non-IgE-mediated antibodies, or a combination of both (Martorell-Aragonés et al. 2015) and ice cream product has a small number of phytochemical content and nutritional benefit. The entrepreneurs then must innovate ice cream and add local fruit and lower the product's energy to make it more competitive (Patil & Banerjee, 2017; Thammasane, 2018). Products that contain no dairy ingredients were further developed, e.g., sherbet, sorbet and yogurt ice cream. Moreover, the trend toward non-dairy ice cream products Utilizing fruit as a raw material to increase the flavor that is preferred by all customer groups (Perera, Weerahewa, & Vidanagamage, 2017). Due to the distinctive flavor of the sour fruit, consumers feel refreshed and keep eating without getting too full (Klee, 2010). A recent study by Kim, Chang, & Yuh (2003) involved study was evaluated characteristics and nutritional analysis of sherbet mulberry ice cream and ice-bar with mulberry fruits in low level of mulberry. The study of high-level mulberry was not study yet and study of variation type of ice cream were not done before.

Therefore, the aim of this work was to examined physical and chemical of mulberry fruits at different maturity stages. To develop and improve the functional properties and nutritional quality of ice cream by utilizing mulberry as an ingredient, researchers are also interested in applying mulberry at two maturity stages in ice cream formulation and explored on sensory characteristic, physical, chemical, nutrition composition and microbiological properties of ice cream.

MATERIAL AND METHODS

Study physical, chemical and nutrition composition of mulberry fruit at the variation of harvesting time

Fresh mulberry was harvested from a mulberry farm in Nakhon Si Thammarat province, Thailand. The mulberry samples were collected from two harvesting times of one-year-old mulberry; there are dark red fruits from the semi-ripened stage (45 days after fruiting) and black-purple fruits from the fully ripened location (52 days after fruiting). The mulberry fruit samples were kept in an aluminum foil bag and stored at -20 °C before being analyzed. The mulberry fruits from two ripened stages were analyzed for physical properties, which are total soluble solid (TSS), pH (AOAC, 2016).

The proximate composition of mulberry including crude protein, total carbohydrate, fat, ash and crude fiber utilizing modified methodologies from AOAC (2016). The phenolic compound (TPC) with Folin–Ciocalteu colorimetric method Ainsworth and Gillespie, total flavonoid content (TFC) aluminum chloride colorimetric assay adapted from (Chatatikun & Chiabchalard, 2013; Roy, Ferdiousi, Khatun, & Moral, 2016) and anthocyanin content (AA) of mulberry fruits were analyzed by AOAC (2016).

Study the effect of mulberry supplementary in mulberry ice cream

This study was conducted using a completely randomized design (CRD). The highest acceptability of the four standard formulations received from the first experiment; then, the mulberry supplementation dosage was used in each mulberry ice cream formulation. Mulberry fruits replaced the water part in the selected standard milk-based ice cream formulation. In the chosen milk-based ice cream formulation, the water part was replaced by mulberry fruits. The fruit part was replaced by mulberry fruits, the selected sorbet, sherbet, milk ice cream and yogurt ice cream. The 9-point hedonic scale was applied for sensory evaluation (1 - 9 = dislike extremely - like extremely) (Stone, Bleibaum, & Thomas, 2020). All ice creams were submitted to a sensory evaluation to evaluate color, flavor, taste, smoothness and overall acceptance of products. The sixty untrained panelists were employed for sensory evaluation.

Mulberry fruits was prepared by combination of semi-ripened mulberry (dark red to purple color) and fully-ripened mulberry (purple to black color) in a ratio of 30:70 (w/w), respectively. The mulberry was rinsed and dewatered before being blended for 30 seconds, 6 times. The combined mulberry was filtered to remove the insoluble parts, then heated to 80 °C and rapidly cooled before being kept in a closed container before ice cream production.

The four mulberry ice creams were produced by adding mulberry 30:70 (w/w) ratio, proportion as shown in Table 1. The mixture was pasteurized (82 °C, 15 second), homogenized (15 MPa, 65 °C) and aged (4 °C, 12 hours) to produce ice cream mixes. The ice cream mixtures were then whipped, frozen for 30 minutes, ice cream kept in the freezer at -18±2 °C for 24 hours to stabilize and retard meltdown of ice cream between servings. These procedures were used to reduce variation in sensory evaluation (Wan et al., 2022; Tansakul, Junden, & Yotmanee, 2022).

The mulberry ice cream was prepared by weight 25 g per scoop and served 1 scoop in 2 ounces clear plastic cup with a cover lid coded at an ice cream cup with a 3-digits number by the white label. Each sample was coded by a 3-digit random number. The panelist received 1 cup of ice cream from 4 different ice cream formulations. Ice cream samples are served after they leave the freezer. The ice cream temperature ranged from -18 to -10 °C. Cleanse the mouth between samples with water into an opaque cup with a cover. Before evaluating the following illustration, the panelists had to rest for a few minutes to bring the palate temperature close to body temperature (Stone, Bleibaum, & Thomas, 2020).

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of WALAILAK UNIVERSITY for studies involving humans. (no. WUEC-21-057-01.)

Study physical, chemical, nutrition composition and microbiological qualities of first and second most accepted mulberry ice cream

The mulberry ice cream formulation, which received the two highest overall acceptability scores from the sensory evaluation, was selected to study physical, chemical, nutrition composition and microbiological qualities.

The viscosity of ice cream mix samples which were aged at 4 °C for 12 hours were evaluated for viscosity using viscometer (Brookfield Engineering Laboratories Inc., United States). The samples were analyzed at 5±1 °C with UL/Y adapter with controlled the speed test at 250 rpm. The results were reported in Pa.s unit.

The pH value of ice cream was investigated and the pH value of both mulberry ice cream was recorded by a pH meter. The C.I.E. color values were measured and expressed as L* (lightness), a* (greenness-redness) and b*

Table 1: Mulberry ice cream formulation

Composition	Mulberry ice cream formulations			
	Mulberry sorbet	Mulberry sherbet	Mulberry milk icecream	Mulberry yogurt icecream
Mulberry fruit (80:20%)	600	640	400	400
Purple color (g)	420	448	120	280
Red color (g)	180	192	280	120
Pasteurized cow milk (g)	-	370.0	1,000.0	320.0
Whipping cream (g)	-	-	500.0	480.0
Sugar (g)	260.0	220.0	220.0	230.0
Dextrose (g)	-	-	60.0	20.0
Glucose (g)	160.0	150.0	-	-
Salt (g)	1.5	1.5	1.5	1.5
Milk based stabilizer (g)	-	-	20.0	20.0
Fruit based stabilizer (g)	20.0	20.0	-	-
Emulsifier (g)	6.0	6.0	4.0	4.0
Water (g)	800.0	350.0	-	-
Plain yogurt (g)	-	-	-	500
1% Citric solution (g)	1.0	1.0	-	-
Total	1,848.5	1,758.5	2,205.5	1,975.5

(Blueness-yellowness). The %Overrun of ice creams was evaluated by the method of Akın, Akın, & Kırmacı (2007). First, the ice cream mix was weighed in a weighted plastic container by a 2-digit balance and recorded ice cream mix weight. Then frozen ice cream was weighed on the same plastic container for the calculated overrun rate as equation 1. Ice cream samples were analyzed after 1 day of storage.

$$\%Overrun = ((Ice\ cream\ mix - Frozen\ ice\ cream))/(Frozen\ ice\ cream) \quad (1)$$

The meltdown rate was analyzed using the modified method from Atalar, Kurt, Gul, & Yazici (2021). The ice cream was frozen at -18 °C and 50 g of ice cream was scooped using an ice cream scooper with a 3-digits balance for recorded initial weight. The ice cream was placed on a wire screen with mesh no. 8 and the container was placed under the wire screen. The meltdown rate of ice creams was measured at 25±2 °C by weighing the dripped ice cream every 10 minutes for 1 hour and calculated rate of meltdown rate using equation 2.

$$Melt\ down\ rate\ per\ 50\ grams = (Dropped\ ice\ cream)/(Initial\ ice\ cream\ weight) \times 100 \quad (2)$$

The nutrition composition of both mulberry ice creams was analyzed nutritional values by the method of AOAC (2016) follows list; pH, energy from fat, total fat, saturated fat, cholesterol, protein content, total Carbohydrate, Dietary fiber, ash, moisture. total sugar using modified method from AOAC (2019), Total energy of mulberry ice cream was calculated using the equation (3).

$$Energy\ (Kcal) = (4 \times carbohydrate\ (g)) + (4 \times protein\ (g)) + (9 \times fat\ (g)) \quad (3)$$

Analyzed vitamins and minerals as followed lists; Vitamin A by modified method from, Vitamin B1 by modified, Vitamin B2, calcium and iron content by modified AOAC (2019). The antioxidant capacity was analyzed by D.P.P.H. radical scavenging activity, phenolic compound by Folin–Ciocalteu colorimetric method by Ainsworth, & Gillespie (2007), insoluble fiber, soluble fiber and anthocyanin content AOAC (2016). Both mulberry ice creams were analyzed for microbial quality at day 0, including total plate count (method of FDA BAM 2001), Pour plate method) (Maturin, 2001), *Escherichia coli* (Feng, P., Weagant, Grant, Burkhardt, & US Food and Drug administration, 2020) *Staphylococcus aureus* (Kerr et al. 2019) and *Salmonella* spp. (Andrews et al., 2007).

Statistical analysis

IBM SPSS Statistics for Windows, Version 21.0 software (IBM Corp., Armonk, NY, USA) was utilized for the experimental statistical analysis. Using analysis of variance (ANOVA) with paired sample T-Test and the post hoc Duncan test, the difference between the mean values was investigated at a significant level of 95%.

RESULTS AND DISCUSSION

Mulberry at different maturity stages showed other physical properties in terms of color. Semi-ripened mulberries had a dark red color (45 days after fruiting) with a sour and slightly bitter taste. The maturity of mulberry is based on fruits color from unripe (green) to ripe (red or black color) (Nayab et al., 2020, Lee and Hwang, 2017). The fully ripened mulberries had a sweet flavor of purple to black color (52 days after fruiting). In-season (February) mulberry fruits had a bigger in size but no significantly different with TPC and antioxidation properties (Punthi, & Jomduang, 2021). The photograph of mulberry from unripe to fully ripe is shown in Fig. 1. From determination, TSS of both mulberry samples were not different. In contrast, fully ripened mulberry had lower acidic content than semi-ripened mulberry (as shown in Table 2), resulting in a sweeter taste in fully ripened mulberries consistent with the research of Singhal, Khan, Dhar, Baqual, & Bindroo (2010).

The proximate composition of mulberry with different maturity stages is shown in Table 3. total carbohydrate, crude protein, and Moisture content increased, whereas crude fiber and ash content decreased. Our findings are consistent with Ercisli & Orhan (2007), who found that mulberry fat content ranged from 0.85 to 1.10%. They also linoleic acid was revealed to become the most abundant fatty acid in mulberry, Yang et al. (2015) reported that linoleic acid had no positive effects on blood pressure in humans followed by palmitic acid. Mahmood, Anwar, Abbas, Boyce, & Saari (2012) reveal that mulberry sugar concentrations increased with the ripening stage. Glucose and fructose are the two principal mulberry fruits that contain sugars (Gecer et al., 2016) and in accordance with Koyuncu, Çetinbas, & Erdal (2014) reported that the ash concentration ranged between 0.12% - 0.36%. This information indicated that fully ripened mulberry should be used because of its potential nutrient.

The variation of phenolic compound in each mulberry sample depended on the maturity of mulberry. Mulberry at the semi-ripened stage had 90 mg of quercetin equivalent/g, which was lower than that from the fully ripened location, 97 mg of quercetin equivalents/g. Xu, Huang, Xu, He, & Wang (2020) reported that phenolic organic substances in mulberry fruits provide antidiabetic ability. The findings were comparable to the study of Nayab et al. (2020), who found that fully ripened mulberry of 4 mulberry genotypes (Black short, Chinese white, black long and Chinese long) had greater TPC than semi-ripened mulberry which consistent with the research of Mahmood (2017). Fully ripened mulberry also had a higher total flavonoid compound contents of 185 mg GAE/g than 179 mg/G.A.E. g in semi-ripened mulberry the results are consistent with the research of Hao (2022). The higher flavonoid corresponded to a high level of anthocyanin; a flavonoid compound responsible for the red-blue pigment in berry fruit. A flavonoid compound is an effective neuroprotectant (Yen et al., 2012). The principal anthocyanin present in the fruit is cya-indin-3-glucoside Xu et al. (2020). According to Lee & Hwang (2017), total flavonoid levels in mulberries decreased after fruiting and gradually increased as the mulberry color changed from purple-red to dark red (from 0.1 to 0.4 g/100 g). The anthocyanin content also increases rapidly as the color changes from purple-red to dark red (changing from 0.0 to 2.0 g/100 g) (Table 4), Nayab et al. (2020) who found that fully ripened mulberry of 4 mulberry genotypes (Black short, Chinese white, black long and Chinese long) had greater TPC than semi-ripened mulberry. Fully ripened mulberry also had a higher total flavonoid compound contents of 185 mg GAE/g than 179 mg/G.A.E. g in semi-ripened mulberry. The higher flavonoid corresponded to a high level of anthocyanin; a flavonoid compound responsible for the red-blue pigment in berry fruit. The principal anthocyanin present in fruit is cyanidin-3-glucoside (Mullen et al., 2002). According to Lee & Hwang (2017), total flavonoid levels in mulberries decreased after fruiting and gradually increased as the mulberry color changed from purple red to dark red (from 0.1 to 0.4 g/100 g). The anthocyanin content also increases rapidly as the color changes from purple red to dark red (changing from 0.0 to 2.0 g/100 g) (Table 4). Overall, fully ripened mulberry has better properties such as higher protein, carbohydrate, total phenolic compound, total flavonoid compound and total anthocyanin content compared to semi-ripened mulberry.

Table 2: Physical composition of Mulberry fruits at different maturity stages

Mulberry fruits	TSS (mg/L)	pH
Semi-ripened stages	35.01±0.01[a]	3.04±0.01[b]
Fully ripened stages	34.00±0.01[b]	3.84±0.01[a]

Mean±SD in a row superscripted with different lowercase letters are significantly ($p<0.05$) different

Effect of mulberry supplement in mulberry ice cream

Mulberry ice cream produced in 4 formulars showed in Fig. 2, Mulberry sorbet has a deep purple color. Next, Mulberry sherbet is slightly less purple due to the milk content. The Mulberry milk ice cream and Mulberry yogurt ice cream are light purple in color. The sensory scores for each mulberry ice cream formulation in each attribute showed in Table 5. The sherbet ice cream obtained the highest score in color, flavor, texture and overall acceptability which were 7.83, 7.00, 7.22 and 7.32, respectively. Panelists gave the highest score for the odor of mulberry sorbet ice cream. Mulberry sorbet and sherbet ice cream texture scores were not significantly different ($p>0.05$). Sorbet and sherbet mulberry ice cream received overall acceptability approximately at 7-8, which are 'Like moderately' to 'Like very much, then it has the probability to apply to commercial products. The mulberry ice cream using higher level of purple (fully ripened) mulberry there are sorbet, sherbet and also yogurt ice cream received the higher sensory score in all attribute which may imply that fully-ripened mulberry influences the panelists' liking scores. Hence, the researcher selected sorbet and sherbet mulberry ice cream to study further.

Study physical, chemical, nutrition composition and microbiological qualities of first and second most accepted mulberry ice cream

The viscosity of ice cream mix, %Overrun, %meltdown and color of mulberry ice cream were shown in Table 6.

Table 3: Proximate composition of Mulberry fruits at different maturity stages

Mulberry fruits	Moisture content (%)	Crude protein (%)	Total Carbohydrate (%)	Fat (%)[ns]	Ash (%)	Crude fiber (%)
Semi-ripened stages	41.00±0.05[b]	1.30±0.04[b]	53.98±0.05[b]	1.02±0.01	0.30±0.11[a]	0.50±0.45[a]
Fully ripened stages	43.00±0.10[a]	1.90±0.01[a]	55.40±0.34[a]	1.00±0.00	0.20±0.10[b]	0.40±1.00[b]

Mean±SD in a row superscripted with different lowercase letters are significantly ($p<0.05$) different, ns: no significant difference

Fig 1. The mulberry fruits range from semi-ripened stage to fully ripened stage.

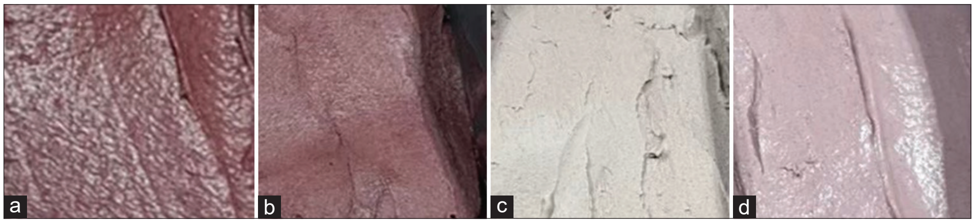

Fig 2. Different type of Mulberry ice cream; Mulberry sorbet (a), Mulberry sherbet (b), Mulberry milk ice cream (c) and Mulberry yogurt ice cream (d).

Table 4: Total phenolic compound, total flavonoid content and anthocyanin content of mulberry fruits at different ripening stages

Sample	Total phenolic compounds (mg GAE/g)	Total flavonoid (mg RUE/100 g DW)	Total anthocyanins (mg/g)
Semi-ripened stages	90.30±0.23^{b}	179.06±0.04^{b}	61.07±0.11^{b}
Fully ripened stages	97.08±0.06^{a}	185.21±0.12^{a}	65.23±0.14^{a}

Mean±SD in a row superscripted with different lowercase letters are significantly (p<0.05) different

Table 5: Sensory evaluation of different type of mulberry ice cream

Attribute	Sorbet	Sherbet	Milk icecream	Yogurt ice cream
Color	6.33±1.66^{c}	7.83±1.04^{a}	6.35±1.58^{c}	7.18±1.66^{b}
Odor	7.30±1.50^{a}	6.38±1.66^{b}	5.18±1.68^{c}	6.42±1.60^{b}
Flavor	7.20±1.70^{a}	7.00±1.74^{a}	4.47±2.18^{b}	6.70±1.69^{a}
Texture	7.56±1.49^{a}	7.22±1.29^{a}	6.52±1.80^{b}	6.16±1.77^{b}
Overall liking	7.20±1.60^{a}	7.32±1.23^{a}	5.18±2.15^{c}	6.63±1.55^{b}

Mean±SD in a row superscripted with different lowercase letters are significantly (p<0.05) different

Table 6: Physical properties of mulberry sorbet and sherbet ice cream

Analysis	Mulberry sorbet icecream	Mulberry sherbet icecream
Viscosity (Pa*s)^{ns}	158.20±0.11	153.80±0.23
%Overrun (gram/100gram)	44.72±1.86^{b}	47.58±1.32^{a}
%Melting down (gram/minute)^{ns}	17.27±1.57	13.81±0.74
Color		
Lightness (L*)	79.85±0.15^{b}	80.85±0.40^{a}
Greenness-redness (a*)	1.45±0.03	1.65±0.13
Blueness- yellowness (b*)	5.83±0.15	6.15±0.08

Mean±SD in a column superscripted with different lowercase letters are significantly (p<0.05) different. ns=not significant

Table 7: Physical properties and nutritional composition of mulberry sorbet and sherbet ice cream

Nutritional composition (Per 100 grams)	Mulberry sorbet ice cream	Mulberry sherbet ice cream
pH	3.32±0.02^{b}	3.70±0.05^{a}
Energy (Kcal/100 g)	113.36±0.02^{b}	132.02±0.15^{a}
Energy from fat (Kcal/100 g)	3.24±0.32^{b}	12.06±0.37^{a}
Total Fat (g/100 g)	0.36±0.01^{b}	1.34±0.05^{a}
Saturated fat (g/100 g)	0.31±0.28^{b}	0.92±0.37^{a}
Cholesterol (mg/100 g)	1.47±0.36^{b}	5.76±0.58^{a}
Protein (g/100 g)	1.32±0.64^{b}	1.48±0.53^{a}
Total Carbohydrate (g/100 g)	26.21±0.15^{b}	28.51±0.53^{a}
Dietary fiber (g/100 g)	0.61±0.11^{b}	0.76±0.24^{a}
Sugar (g/100 g)	19.50±0.05^{a}	18.31±0.09^{b}
Sodium (mg/100 g)	44.987±0.47^{b}	55.820±0.51^{a}
Vitamin A (µg/100 g)	ND	ND
Vitamin B1 (mg/100 g)	ND	ND
Vitamin B2 (mg/100 g)	ND	ND
Calcium (mg/100 g)	59.463±0.03^{a}	87.110±0.29^{b}
Iron (mg/100 g)	0.355±0.07^{b}	0.434±0.05^{a}
Ash (g/100 g)	0.30±0.02^{b}	0.48±0.03^{a}
Moisture content (g/100 g)	71.81±0.15^{a}	68.19±0.23^{b}
Brix (°brix)	27±0.00b	30±0.00^{a}
Insoluble dietary fiber (g/100 g)	0.34±0.49^{b}	0.42±0.10^{a}
Soluble dietary fiber (g/100 g)	0.27±0.21^{b}	0.34±0.38^{a}
Total Phenolic (as Gallic acid) (mg GAE/g)	3.48±0.03^{b}	3.85±0.04^{a}

Mean±SD in a column superscripted with different lowercase letters are significantly (p<0.05) different. ND: Not Detected

Both mulberry ice cream formulas had the same viscosity range. The %Overrun of sorbet mulberry ice cream was lower than sherbet mulberry ice cream due to cow's milk. The development of %Overrun is related to milk protein (Goff, & Hartel, 2013). Because sorbets do not contain milk protein, less air is integrated into the ice cream, resulting in a lower percent overrun. In contrast, sherbet ice cream formulations do, resulting in a more significant %Overrun. Sorbet mulberry ice cream melted faster than sherbet mulberry ice cream. The study of Sakurai (1996) stated that ice cream with lower overruns had a faster melting rate, similar to this experiment's result. The color of both ice creams was similar light-purple color from the red-purple color of anthocyanin in mulberry fruits. The sherbet mulberry ice cream was lighter (higher L*) than sorbet in ice cream. The lighter (L*) of sherbet ice cream was due to the protein content in the product. Incident light is caused by milk fat particles, increasing L* value (Balthazar et al., 2015).

Both mulberry ice creams had low pH, which is an acidic range. However, the sorbet has more acidity than the sherbet ice cream due to the low pH of mulberry and absence of milk, whereas the sherbet ice cream showed higher pH because of the neutral pH of milk in the formulation. Barnes, Harper, Bodyfelt, & McDaniel (1991)

Table 8: Microbial content of sorbet mulberry ice cream and sherbet mulberry ice cream

Type of pathogen	Mulberry sorbet icecream	Mulberry sherbet icecream	Requirement *
Total Plate Count (CFU/g)	<250 EAPC**	<250 EAPC	600,000 CFU/g
Staphylococcus aureus (CFU/g)	<10	<10	not be detected in 0.01 g. of food
Coliforms Bacteria (MPN/g)	<3	<3	not be detected in 0.01 g. of food
Escherichia coli (/0.01g)	Not Detected	Not Detected	not be detected in 0.01 g. of food
Salmonella spp. (/25g)	Not Detected	Not Detected	not be detected in 0.01 g. of food

*Requirement from notification of the Ministry of Public Health No. 354 (B.E.2013) (Ministry of Public Health, 2020) and No. 416 (B.E. 2020) (Ministry of Public Health, 2013)
**EAPC = Estimated aerobic plate counts (Ministry of public health, 2013; Ministry of Public Health, 2020)

reported that higher titratable acidity values in a typical fruit negatively relate to flavor and consumer acceptance. This observation explains why the sorbet ice cream sample had a lower overall acceptance.

Higher nutritional values in several compositions were related to cow milk in sherbet mulberry ice cream, wherewith cow milk is high in protein, lactose (milk sugar), fat and a good source of calcium. These are resulting in higher energy, energy from fat, total fat, saturated fat, cholesterol, protein, total carbohydrates and calcium compared to sorbet formulation. Mulberry sherbet ice cream with a higher fat content has a creamier texture, affecting the ice cream's taste and leading to a higher overall acceptance score. The trace amount of sodium and iron in milk (Flynn, 1992) results in higher sodium and iron in sherbet formulation. The high iron content in mulberry sherbet ice cream is also from the high iron content in mulberry fruit (Adeel et al., 2011).

Fiber added to food systems can provide food products functional properties and health benefits (Soukoulis, Lebesi, & Tzia, 2009). The dietary fiber of sorbet is higher due to the larger ratio of mulberry in ice cream formulation, which is beneficial for health. An absence of vitamin A, B1 and B2 levels was observed in both mulberry sorbet ice cream and mulberry sherbet ice cream. The abundance of dietary fiber in mulberry fruits is suggested to decrease hepatic lipogenesis and increase LDL-receptor function (Thebaudin, Lefebvre, Harrington, & Bourgeois, 1997).

In Table 7, sherbet mulberry ice cream had a higher total solid content (°brix). The milk and a more excellent mulberry ratio in sherbet mulberry ice cream resulted in a higher dry matter content. As a result, they lowered the moisture content in sorbet ice cream. The higher percentage of mulberry also showed a higher level of both insoluble and soluble dietary fiber. Although regular ice cream contains no fiber, supplementing with fiber provides a wide range of health benefits (Thebaudin, Lefebvre, Harrington, & Bourgeois, 1997). The fibers in mulberry are both soluble (25%) in the form of pectin and insoluble (75%) in the form of lignin (Sakagami et al., 2007; Imran, Khan, Shah, Khan, & Khan, 2010) Which can enhance the viscosity of ice cream mix and improve ice cream texture (Tsevdou et al., 2019). pectin extracted from mulberry fruit has the potential for the treatment of bacterial and cancerous illnesses (Kumar et al., 2020). Antioxidant capabilities have been identified in each and every form of lignin (Sugiarto, Leow, Tan, Wang, & Kai, 2022).

In comparison to sorbet mulberry ice cream, sherbet mulberry ice cream had a higher phenolic content. Mulberry is a rich source of phenolic compounds; adding mulberry to ice cream can enhance the phenolic compounds and provide the ice cream's functional characteristics. Mulberry fruit contains quercetin, which is a flavonoid with antioxidant activity. Quercetin prevents blood clots and also reduces the risk of high blood pressure and heart disease.

To ensure food safety, the study of microbial content was conducted, as shown in Table 8. Before being examined, the mulberry ice cream samples were stored at -16±2 °C. Both mulberry ice creams have met the criteria for Total Plate Count, *Staphylococcus aureus*, Coliforms Bacteria, *Escherichia coli* and *Salmonella* spp. (/25g) from notification of the Ministry of Public Health no.354 (B.E. 2556) (Ministry of public health, 2013) in titled ice cream (Ministry of Public Health, 2020) and No.416 (B.E. 2563) (Ministry of Public Health, 2020) in titled Prescribing the quality or standard, principles, conditions and methods of analysis for pathogenic microorganisms in foods (Ministry of Public Health, 2013) (Ministry of public health, 2013). The APC of all ice cream samples also met criteria of the CGMP & PC rule (European Commission, 2005). All of the mulberry ice creams were sanitized and safe for consumers.

CONCLUSIONS

Mulberry is a fruit with a variety of phytochemicals. Total phenolic compound, total flavonoid content and anthocyanin content levels were higher in fully ripened mulberries, which provided health benefits to ice cream consumers. Fruits are regularly added to ice cream products to give a unique flavor. Supplementary of mulberry into sherbet ice cream received significantly highest overall acceptability score followed by sorbet ice cream. The presence of milk impacted the physical properties and nutritional composition of mulberry sherbet ice cream. The phytochemical compositions remain in mulberry sorbet and sherbet ice cream, which could be utilized to increase the functional properties of ice cream without compromising the ice cream quality. Mulberry sherbet ice cream received the highest overall acceptability sensory scored particularly in color attribute. This formulation is safe to consume and recommended to be further manufactured on an industrial scale.

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Authors' contributions

Duangkamon Sangteerakij assisted in conducting the experiments, performed the statistical analysis and data visualization and wrote the manuscript. Sauwanee Rodyu wrote the manuscript. Sirisopa Junden designed and completed all of the experiments and wrote the manuscript. All authors have read and approved the final manuscript.

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