

Research Article

Consumption of Anthocyanin-Rich Mulberry Fruit Jelly with a High-Fat Meal Decreases Postprandial Serum Cardiometabolic Risk Factors in Dyslipidemia Subjects

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Anthocyanin content in berries has been reported to promote antioxidant properties that mitigate the occurrence of non-communicable diseases. However, only a few studies have investigated the benefits of anthocyanin-rich food products from mulberry fruit to reduce the cardiometabolic risk factor in dyslipidemia subjects. Anthocyanin-rich mulberry fruit jelly was formulated using mulberry fruit powder (MFP), and its activities on serum cardiometabolic risk factors in dyslipidemia subjects were studied. *Morus alba* var. Chiang Mai was used as the ingredient for MFP jelly containing 14 g MFP (191 mg anthocyanin) per serving size (170 g). To investigate the effect of MFP jelly on reduction of cardiometabolic risk factors, sixteen dyslipidemia subjects were given one serving of MFP jelly every day for seven days. After MFP jelly intervention, fasting blood cholesterol, low-density lipoprotein (LDL), and inflammatory markers including interleukin-6 levels of the subjects were significantly lower. Postprandial blood parameters were measured at 0–240 min after consuming a high-fat meal before and after MFP jelly intervention. Postprandial blood glucose at 30 min ($p < 0.05$) and insulin at 60 and 90 min ($p < 0.01$) were lower in MFP than in placebo jelly. The area under the curve of insulin in MFP jelly was smaller than in placebo by 31.2%. Therefore, MFP jelly intervention increased insulin sensitivity. For antioxidant activity markers, postprandial oxygen radical absorbance capacity after MFP jelly intervention gave a smaller decrease after high-fat meal intake compared to after placebo jelly intervention. Moreover, for the oxidative stress markers, postprandial malondialdehyde level was significantly lower in MFP jelly. Seven days of intervention by one serving size of MFP jelly containing 191 mg of anthocyanins reduced cardiometabolic risk factors by lowering blood total cholesterol, LDL, and inflammation, and improving insulin sensitivity and postprandial blood antioxidant-oxidative stress activity in dyslipidemia subjects. This trial is registered with TCTR20200415003.

1. Introduction

Cardiometabolic risk factors refer to conditions that increase the risk of vascular disease and diabetes exposure. These conditions can be clustered as metabolic syndrome and include abdominal obesity, hypertension, hyperglycemia,

elevated triglyceride and high-density lipoprotein (HDL) cholesterol, in addition to low-density lipoprotein (LDL) cholesterol, insulin resistance, smoking, and inflammatory markers [1]. Increased oxidative stress has been associated with various cardiometabolic risk factors [2]. Overnutrition such as excessive glucose, lipid, and caloric intake induce

free radical increase during the postprandial period. Lipid peroxidation from polyunsaturated fatty acids and oxidized by free radicals produce malondialdehyde (MDA) as a by-product. Increased MDA has been reported in people with obesity, metabolic syndrome (MetS), and type 2 diabetes mellitus (T2DM) [3]. More intense increased oxidative damage manifests as hyperlipidemia and hyperglycemia that affect antioxidant status [4]. Polyphenol-rich fruit intervention and postprandial studies in humans have also shown protective effects against oxidative stress and inflammation [5].

Mulberry fruit (*Morus alba*) contains vitamins, polyphenols (including flavonoids, anthocyanins, and carotenoids), carbohydrates, fiber, minerals, riboflavin, ascorbic acid, nicotinic acid, and essential fatty acids [6–8]. These compounds have been recognized for their health-related properties that include antihyperglycemic [9], hypolipidemic [10], antioxidant [11], and anti-inflammatory activities [12]. Studies of anthocyanin-rich berries have reported protective effects on cardiometabolic risk factors with various intervention periods. A 599 mg dose of anthocyanin was reported to affect postprandial blood glucose without any change at lower doses of 132 and 320 mg [13]. Intake of blackcurrant powder with 138.6 mg of anthocyanin by seventeen healthy participants was observed for seven days, and results showed reduced glucose and insulin response [14]. However, there are no reports concerning the benefits of anthocyanin-rich mulberry fruit products on cardiometabolic risk factors in dyslipidemia subjects.

Therefore, functional food products from mulberry to reduce the risk of cardiometabolic disorders should be investigated. Jelly is a favorite dessert among all age groups, and the process of making jelly preserves ripe fruit, adding value to the final product. Mulberry fruit powder (MFP) jelly was formulated with consumer acceptability and used to examine the effects of repeated intake over a period of seven days on cardiometabolic risk factors. Postprandial blood antioxidant, glucose and lipid levels, and inflammatory markers were measured together with fasting in dyslipidemia subjects.

2. Materials and Methods

2.1. Human Study. This study involved human subjects and was conducted according to the guidelines laid down in the Declaration of Helsinki. All procedures involving human subjects were approved by the Institutional Review Board. This protects the rights and welfare of human subjects in research studies conducted under the auspices of the Mahidol University Central Institute Review Board (COA no. MU-CIRB 2018/139.1007). All subjects gave written informed consent before participation.

2.2. Development of Anthocyanin-Rich Mulberry Fruit Jelly Using Thai Mulberry Fruit Powder

2.2.1. Raw Materials. Mulberry fruit (*Morus alba* var. Chiang Mai) was obtained from Kamnulchul Farming Co., Ltd., Thailand. Fully mature fruits were freeze-dried to

obtain mulberry fruit powder (MFP) and kept in vacuum aluminum foil bags at -20°C until required. Other ingredients used for jelly making included carrageenan, locust bean gum, konjac, sucralose, and citric acid (Chemipan Corporation Co., Ltd., Thailand).

2.2.2. Preparation of Anthocyanin-Rich Mulberry Fruit Jelly.

The jelly consisted of two different textures as soft and hard. Ingredients of anthocyanin-rich mulberry fruit jelly are shown in Table 1. Jelly was prepared as presented in the flowchart (Figure 1). First, the hard texture jelly was prepared by mixing gelling agents and water. The mixture was heated and stirred at 80°C for 20 min until it became clear. Mulberry fruit powder (MFP) was then dissolved in water and added into the clear solution together with citric acid and sucralose. The dispersion was reheated for approximately 3 min at 80°C . To obtain a desirable gel consistency, the endpoint was judged when the yield of viscous dispersion reached 100 g. The dispersion was then poured into a square mold and cooled to 4°C for 24 h. The hard texture jelly was cut into cubic shapes of size $15 \times 15 \times 10$ mm. Then, the soft texture jelly was prepared using a similar method. Finally, 50 g of hard texture jelly was added into the same mold dispersion as the soft texture jelly (120 g) and left to set at 4°C for 24 h.

2.2.3. Nutritive Values. Moisture content, ash, protein, fat, carbohydrate, insoluble and soluble dietary fiber, and sugar were determined using the standard method of AOAC (2016) [15]. Caloric content was calculated based on the contents of protein, fat, and carbohydrate.

2.2.4. Total Phenolic Contents and Antioxidant Activities.

Jelly samples were lyophilized using a Heto Powerdry PL9000 freeze dryer (Heto Lab Equipment from Allerod, Denmark) at -80°C for 48 h. Dried jelly samples were homogenized into powder using a blender (TEFAL 400 Watt from Tefal Co., Ltd., France) and stored in vacuum aluminum foil bags at -20°C . The extraction method was performed by mixing approximately 50 mg of the sample with 1 mL of 70% MeOH (methanol). After thoroughly mixing (1 min) with a vortex mixer, the mixture was heated in a water bath at 50°C for 2 h. The supernatant was collected using a 4°C centrifuge (model Hettich ROTINA 38R, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany) at 4,600 rpm for 15 min. The supernatant was then filtered through a $0.45 \mu\text{m}$ PES filter and kept at -20°C until required for analysis. Total phenolic contents (TPCs) were determined using the Folin–Ciocalteu reagent and expressed as gallic acid equivalents per gram dry weight (mg GAE/g DW) of the sample [16, 17]. Antioxidant activities were determined using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, oxygen radical absorbance capacity (ORAC), and ferric ion reducing antioxidant power (FRAP) assays [18–20]. Results were presented as Trolox equivalents per gram dry weight (TE/g DW).

TABLE 1: Amount of ingredient (%) in the anthocyanin-rich mulberry fruit powder (MFP) jelly.

Ingredients	MFP jelly	
	Soft texture	Hard texture
Carrageenan	0.33	1.20
Locust bean gum	0.5	1.40
Konjac powder	0.08	0.80
Sucralose	0.0125	0.01
Citric acid	0.17	0.05
MFP	2.5	22.00
Water	96.41	74.54

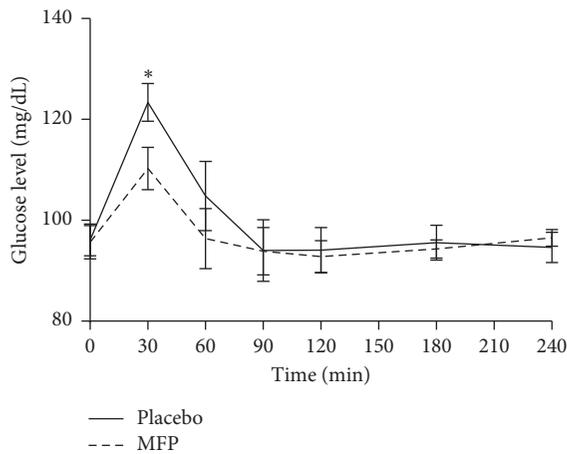


FIGURE 1: Blood glucose response after consuming two jelly samples (placebo and MFP jelly), data expressed as mean \pm SE. The sign “*” expressed as statistically significant (p value < 0.05).

2.2.5. *Anthocyanins.* Identification of anthocyanins and anthocyanidins was performed using HPLC analysis as indicated by Suttisansanee et al., 2020 [21]. Anthocyanin standards including cyanidin-3-O-glucoside (kuromanin), cyanidin-3-O-rutinoside (keracyanin), cyanidin-3,5-O-diglucoside (cyanin), cyanidin-3-O-galactoside (idaein), pelargonidin-3,5-O-diglucoside (pelargonin), malvidin-3-O-galactoside (primulin), and petunidin-3-O-glucoside, and anthocyanidin standards including cyanidin, delphinidin, pelargonidin, peonidin, petunidin, and malvidin were purchased from Sigma-Aldrich (St. Louis, MO, USA). The existence and contents of anthocyanins and anthocyanidins were identified by comparing their retention times (Rts) and UV-vis spectral fingerprints with the standards.

2.2.6. *Sensory Evaluation.* A nine-point hedonic scale (1 = dislike extremely to 9 = like extremely) was chosen to conduct the sensory evaluation of the jelly samples. In addition, a just-about-right scale (1 = too little, 3 = just about right, and 5 = too much) was used to consider the intensity of specific attributes of the jelly as sweetness and sourness. A panel of thirty judges evaluated the product for sensory parameters including appearance, color, odor, flavor, texture, and overall acceptability. Ten grams of MFP jelly was served in capped transparent plastic cups [22].

2.3. Effect of Anthocyanin-Rich Mulberry Fruit Powder (MFP) Jelly Intervention over a Period of Seven Days on Postprandial Blood Cardiometabolic Risk Factors in Dyslipidemia Subjects: An Open Label Trial of MFP Jelly Intervention with Prepost Analysis

2.3.1. *Subjects and Study Design.* An open label trial was conducted with a seven-day intervention and prepost analysis. Fifteen dyslipidemia volunteers (total cholesterol > 200 mg/dL; LDL cholesterol > 130 mg/dL; HDL cholesterol < 40 mg/dL (men) and < 50 mg/dL (women); triglycerides > 150 mg/dL) participated (Figure 1). Exclusion criteria included body mass index (BMI) > 35 kg/m², smokers, athletes with high physical activity ($> 3,000$ kcal/day), diabetes, multiple allergies, intestinal diseases, or any other disease or condition that would worsen adherence to the measurements or treatment.

2.3.2. *Test Meal.* A high-fat meal with 652 kcal contains 32% of fat distribution for energy per day. The test meal consisted of shredded pork with a salad cream croissant sandwich, two pieces (75 g) of chicken frank sausages, and 250 mL of a sweetened beverage. Energy distribution of carbohydrate: protein: fat was 29:11:60. Nutrient composition of the high-fat meal was analyzed by INMUCAL-nutrients computer program version 3 (INMU, Thailand).

2.3.3. *Blood Collection.* Blood samples (10 mL) were collected intravenously by a registered nurse. Blood glucose, insulin, lipid, and antioxidant were taken at 0 (baseline), 30, 60, 90, 120, 180, and 240 min after the first ingestion of the high-fat meal followed with MFP jelly. Blood samples were centrifuged at 3,500 rpm for 15 min. Plasma and serum were collected into vials and stored at -80°C until required for further study.

2.3.4. *Blood Chemistry.* Blood glucose, insulin, and lipid profiles including triglyceride, total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were analyzed at Bangkok Medical Lab Co., Ltd.

2.3.5. *Plasma Antioxidant, Oxidative Stress, and Inflammatory Markers.* Oxygen radical absorbance capacity (ORAC) assay was measured from plasma using EDTA as an anticoagulant. Plasma was diluted with 0.1 M phosphate buffer saline. The next step of protocol followed the method previously described by Ou et al. [19]. Results were reported in $\mu\text{mol TE/L}$. Malondialdehyde (MDA) assay was adapted from [23]. The sample was mixed thoroughly in a vortex mixer for 30 seconds with 20% trichloroacetic acid (TCA) at ratio 1:1 before incubation at room temperature for 15 min. The mixture was then centrifuged at 12,000 g for 4 min. The supernatant was mixed with thiobarbituric acid (TBA) 0.8 (w/v) before boiling at 100°C for 30 min. The assay was monitored at excitation wavelength of 530 using the microplate reader. The MDA value was calculated and expressed in nmol/L. Inflammatory markers such as IL-6

were measured by enzyme-linked immunosorbent assay using paired antibodies purchased from eBioscience Inc. (San Diego, CA, USA).

2.4. Statistical Analysis. Data were presented as mean \pm SD. Bioactive compounds and antioxidant activities of the control and MFP jelly were subjected to Student's t-test. Fasting blood glucose, insulin, and lipid profiles at before and after MFP jelly intervention were analyzed by the paired t-test. Area under the curves of changes in insulin, triglyceride, and glucose were calculated using the repeated measure ANOVA. Changes in area under the curves (AUCs) before and after intervention were analyzed by the paired t-test. Calculations and statistical analyses were conducted using SPSS version 19 (Chicago, USA). Statistical significance was accepted at p value <0.05 .

3. Results

3.1. Development of Anthocyanin-Rich Jelly Using Thai Mulberry Fruit Powder

3.1.1. Sensory Evaluation. Anthocyanin-rich MFP jelly (170 g) included 120 g of soft jelly and 50 g of hard jelly. Table 2 shows the sensory evaluation scores of MFP jelly as assessed by 30 untrained panelists. MFP jelly obtained sensory scores in the range of 7.67–7.83 (like moderately to like very much). The 5-point just-about-right scale also indicated that sweetness and sourness intensities of MFP jelly were at just about right.

3.1.2. Nutritive Values of MFP Jelly. Nutritional values were reported in terms of energy, moisture, protein, total fat, total carbohydrate, dietary fiber (soluble and insoluble), total sugar, and ash of the MFP jelly (Table 3). The energy, protein, carbohydrate, dietary fiber (soluble and insoluble), and sugar were 66.16, 1.84, 14.71, 4.20, 2.74, and 4.56 g per 170 g, respectively. However, no fat content was found in the product. Moreover, the percent daily values of MFP jelly were calculated based on a 2,000 calories diet, 50 g of protein, 65 g of total fat, 300 g of total carbohydrate, 25 g of dietary fiber, and 25 g of sugar were 3, 4, 0, 5, 17, and 7%, respectively.

3.1.3. Anthocyanins and Phenolic Compounds and Antioxidant Activities of MFP Jelly. Table 4 shows the anthocyanins and phenolic compounds and antioxidant activities of the control and MFP jelly. Results found that MFP jelly contained significantly higher total phenolic contents (TPCs) and antioxidant activities than the control jelly. In addition, no anthocyanins were found in the control jelly. TPCs, antioxidant activities by DPPH radical scavenging, FRAP and ORAC assays, and total anthocyanin of MFP jelly were 21.34 mg GAE/g DW, 1.20 μ mol TE/100 g DW, 127.68 μ mol TE/100 g DW, 67.77 μ mol TE/100 g DW, and 12.76 mg/g DW, respectively (Table 4). Moreover, results showed that MFP jelly consisted of cyanidin-3-glucoside (C3G), cyanidin-3-rutinoside (C3R), and cyanidin at 7.40 mg/g DW,

5.36 mg/g DW, and 1.31 μ g/g DW, respectively (supplementary Figures 1 and 2). According to the calculation, MFP jelly contained 191 mg of anthocyanins per serving size (170 g).

3.2. Effect of Seven Days of Intervention by Anthocyanin-Rich Jelly Made from Mulberry Fruit Powder (MFP) on Postprandial Blood Cardiometabolic Risk Factors in Dyslipidemia Subjects: An Open Label Trial Using MFP Jelly Intervention with Prepost Analysis

3.2.1. Characteristics of Subjects at the Baseline. Fifteen dyslipidemia subjects ($n=15$) were observed. There were seven males with an average age of 32.6 years. Mean and standard deviation of the body mass index was 23.46 ± 3.26 kg/m². After seven days of intervention with MFP jelly (191 mg of anthocyanin/day), the BMI of subjects did not change. Also, energy intake and macronutrient intake did not change during the period before and after seven days of MFP jelly intervention. Saturated fatty acid intake was significantly higher during the MFP jelly intervention period (data not shown).

3.2.2. Effect of MFP Jelly after Seven Days of Intervention on Blood Cardiometabolic Risk Factors. After seven days of MFP jelly intervention, blood lipid as cholesterol and LDL significantly decreased by 7% and 10% with decreasing p values at 0.003 and 0.001, respectively. In terms of inflammatory markers, the IL-6 level was lower after 7 days of MFP jelly consumption (Table 5).

3.2.3. Effect of MFP Jelly Intervention after High-Fat Meal Consumption on Postprandial Blood Glucose, Insulin, and Lipid Profiles. At one day before and after seven days of MFP jelly intervention, postprandial blood glucose, insulin, and triglyceride were observed after the consumption of a high-fat meal. All subjects ingested a 170 g placebo jelly (on the day before MFP jelly intervention) and a 170 g MFP jelly (on the day after seven days of MFP jelly intervention). The placebo jelly contained 10 g of available carbohydrate which was the same amount as the MFP jelly. Comparison between the placebo and MFP jelly groups showed that blood glucose level was significantly lower in the MFP jelly group at 30 min (p value = 0.027) (Figure 1). Mean insulin level in the MFP group was significantly lower when compared to the placebo group at 60 and 120 min (p values = 0.001 and 0.002, respectively) (Figure 2). Interestingly, when insulin sensitivity was analyzed by the AUC calculation, the MFP jelly group generated smaller AUC than the placebo jelly group by 31.22% with statistical difference (p value <0.001). The effect of MFP jelly on postprandial triglyceride was also observed. Triglyceride showed an increase after 120 min and decline after 240 min in both jelly groups. However, no statistically significant difference was observed (Figure 3).

TABLE 2: Sensory evaluation scores of MFP jelly¹.

Sensory attribute	Scores
Appearance ²	7.77 ± 0.94
Color ²	7.83 ± 0.79
Odor ²	7.73 ± 0.78
Taste ²	7.67 ± 0.92
Texture ²	7.70 ± 0.84
Overall liking ²	7.80 ± 0.85
Sweetness ^{3,ns}	2.77 ± 0.68
Sourness ^{3,ns}	2.97 ± 0.41

¹Results are mean ± standard deviation (SD) ($n = 30$). ²Nine-point hedonic scale (1 = dislike extremely and 9 = like extremely). ³5-point just-about-right scale (1 = too little, 3 = just about right, and 5 = too much). ^{ns}No significant difference with 3 (just about right) at $p < 0.05$ by one-sample t-test.

TABLE 3: Nutritive values and % dairy values of MFP jelly.

Nutritive values (170 g/serving)	MFP jelly	% dairy values ¹
Energy (kcal)	66.16	3
Protein (g)	1.84	4
Total fat (g)	0.00	0
Total carbohydrate (g)	14.71	5
Dietary fiber (g)	4.20	17
Soluble dietary fiber (g)	2.74	
Insoluble dietary fiber (g)	1.46	
Total sugars (g)	4.56	7
Moisture (%)	152.15	
Ash (g)	0.95	

¹Percent daily values are based on a 2,000 calories diet.

3.2.4. Effect of MFP Jelly Intervention after High-Fat Meal Consumption on Postprandial Antioxidant Activity and Oxidative Stress. Seven days of MFP jelly intervention showed a strong effect on changes in postprandial blood oxygen radical absorbance capacity (ORAC) levels (Figure 4). Antioxidant activity levels from ORAC assays decreased in both placebo and MFP jelly groups after the high-fat meal consumption. A postprandial change from baseline was observed in both groups. After 90 min, the MFP jelly intervention group showed a smaller decrease in antioxidant ORAC level than the placebo group (p value < 0.05). In the analysis of oxidative stress by the MDA concentration (MDA) assay (Figure 5), MDA level increased after high-fat meal consumption in both groups. However, the MFP jelly group showed a smaller increase in MDA level at all time points compared to the placebo group.

Your daily values may be higher or lower depending on your calorie needs. Protein, total fat, total carbohydrate, dietary fiber, and sugar are not less than 50 g, 65 g, 300 g, 25 g, and 25 g, respectively.

4. Discussion

MFP jelly containing 14 g MFP (191 mg anthocyanin) per serving size (170 g) was acceptable by the consumer. Moreover, addition of sucralose and citric acid was at the optimal amount according to results of sweetness and sourness intensity that were at just about right. This result was expected since sucralose is a nonnutritive sweetener

with no effect on bitter aftertaste, while citric acid can help to suppress bitter taste [24, 25]. Therefore, MFP jelly was used to demonstrate the effect of the product on postprandial cardiometabolic risk factors in dyslipidemia subjects. According to the Notification of the Ministry of Public Health No.182 (B.E.2541) on nutritional labeling, MFP jelly could be claimed as a low-calorie product as the energy content was lower than 40 kcal per 100 g solid food. This result was due to the replacement of sucralose in the product. Sucralose tastes like sugar, has no unpleasant or bitter aftertaste, and is noncaloric, noncarcinogenic, and proven safe for human consumption [26, 27]. Moreover, MFP jelly could also be claimed to be rich/high fiber since the amount of fiber is higher than 6 g per 100 kcal. This was because the addition of MFP is also rich in fiber [28]. MFP jelly also contains protein. Previous researchers found that mulberry contained higher protein than strawberry and sunberry [29, 30]. Total sugar was lower than 5 g per 100 g; therefore, MFP jelly is low sugar as classified by the front of pack labels. Increase of bioactive compounds and antioxidant activities of MFP jelly could be due to the addition of MFP which is a source of antioxidant and anthocyanin [11]. Moreover, the amount of TPCs and antioxidant activities depend on the processing condition. Conditions that retain higher content of polyphenols and high antioxidant activity involve relatively low moisture ($< 14\%$) and temperatures ($< 140^\circ\text{C}$) [31–33]. Several studies reported health benefits related to TPCs and their antioxidant activity such as antihyperglycemic [9], hypolipidemic [10], and anti-inflammatory [12] activities in both in vivo and in vitro studies. Therefore, MFP jelly would be advantageous as a functional food product with high levels of these bioactive compounds to provide health benefits to consumers.

This study observed dyslipidemia individuals for the effects of a seven-day intake of anthocyanin-rich MFP jelly on fasting glucose, insulin, lipid profiles, and inflammatory markers, as well as postprandial glucose and insulin, antioxidant capacity, and oxidative stress level after a high-fat meal intake. After seven days of MFP jelly intake, fasting total cholesterol, LDL cholesterol, and IL-6 significantly decreased as well as postprandial glucose, insulin, and antioxidant capacity from ORAC assay and MDA decreased. Results showed that MFP jelly could be used as a functional food product to reduce blood cardiometabolic risk factors in hypercholesteremic subjects.

The health benefits of MFP jelly result from the bioactive compounds or nutrients in the product, especially anthocyanins in mulberry fruit. Anthocyanin is the major bioactive compound in mulberry fruit and consists of C3G and C3R that have been reported to prevent noncommunicable diseases [9, 10, 12]. However, differences in dose and subject characteristics have been observed. Seven days of acute intake with a crossover study design indicated that low-dose intake of total anthocyanins (138.6 mg) from blackcurrant powder in healthy participants reduced postprandial glucose and insulin response comparable in this study, but no reports on lipid profiles, oxidative stress, and inflammatory markers were presented [14]. Twenty-seven overweight or obese men were given 600 g of frozen blackberries (361 mg

TABLE 4: Anthocyanins and antioxidant activities of MFP jelly¹.

Anthocyanins and antioxidant activities	Jelly formulas*	
	Control	MFP
TPCs (mg GAE/g DW)	2.93 ± 0.12	21.34 ± 0.71
Antioxidant activities	DPPH radical scavenging assay (μmol TE/100g DW)	0.17 ± 0.02
	FRAP assay (μmol TE/g DW)	4.11 ± 0.39
	ORAC assay (μmol TE/g DW)	ND
Total anthocyanin (mg/g DW)	ND	12.76 ± 3.80
Cyanidin-3-glucoside (C3G) (mg/g DW)	ND	7.40 ± 2.23
Cyanidin-3-rutinoside (C3R) (mg/g DW)	ND	5.36 ± 1.57
Cyanidin (μg/g DW)	ND	1.31 ± 0.17

¹Results are mean ± standard deviation (SD) ($n = 3$); ND = not detected. *Samples were extracted with 70% MeOH (50 mg/mL). The extracts were shaken at 50°C for 2 h to reach the optimized extraction of TPCs, antioxidant activities, and total anthocyanin and cyanidin contents. TE = trolox equivalent; GAE = gallic equivalent; Dw = dry weight.

TABLE 5: The biochemical parameters of subjects at 1st (before MFP jelly intervention) and 2nd (after MFP jelly intervention) study visit^{1,2}.

Parameters	Before	After	<i>p</i> value
Systolic blood pressure (mmHg)	116.81 ± 19.81	111.44 ± 10.61	0.051
Diastolic blood pressure (mmHg)	70.44 ± 9.34	69.81 ± 8.47	0.705
Pulse (time/min)	75.64 ± 13.55	77.38 ± 14.04	0.440
Glucose (mg/dL)	96.13 ± 12.60	95.68 ± 13.23	0.821
Insulin (uU/L)	9.92 ± 3.48	8.56 ± 2.64	0.052
Triglyceride (mg/dL)	104.31 ± 35.03	111.69 ± 53.56	0.383
Cholesterol (mg/dL)	229.44 ± 26.58	214.38 ± 21.61	0.003
High-density lipoprotein (mg/dL)	54.25 ± 13.14	52.88 ± 13.87	0.253
Low-density lipoprotein (mg/dL)	154.13 ± 19.80	138.69 ± 18.26	0.001
Interleukin-6 (pg/mL)	555.11 ± 436.58	517.71 ± 444.76	0.027

¹Results are mean ± standard deviation (SD).²*p* value on the paired t-test at $p < 0.05$.

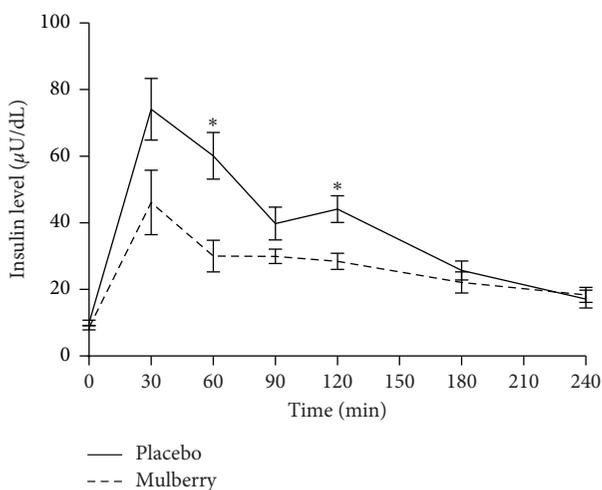


FIGURE 2: Blood insulin level after consuming two jelly samples (placebo and MFP jelly), data expressed as mean ± SE. *Statistically significant (p value < 0.05).

anthocyanin) for seven days, compared with 191 mg in this study [34]. Results showed a significant difference in insulin response, whereas no significance was seen in blood glucose and triglyceride changes. Moreover, this study was interested in short-term anthocyanin-rich berries and postprandial inflammation markers after high-fat meal intake. A

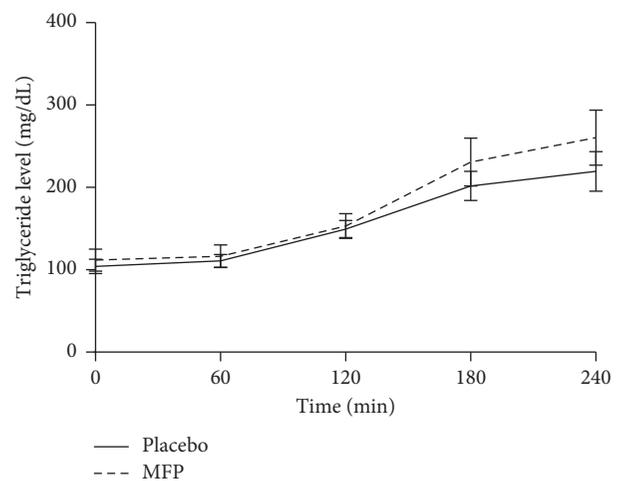


FIGURE 3: Blood triglyceride level after consuming two jelly samples (placebo and MFP jelly), data expressed as mean ± SE. *Statistically significant (p value < 0.05).

four-day crossover consumption of 45 g/d of lyophilized black raspberries (BRB) with and without high-fat, high-calorie meal (HFHC) was reported in elderly and overweight or obese men. The AUC of IL-6 was significantly lower in BRB with HFHC than BRB alone; however, no significant changes in the AUC of CRP and TNF- α [35]. Another longer 24-week study was performed on type 2 diabetes patients who received 160 mg of anthocyanins twice daily (mixed

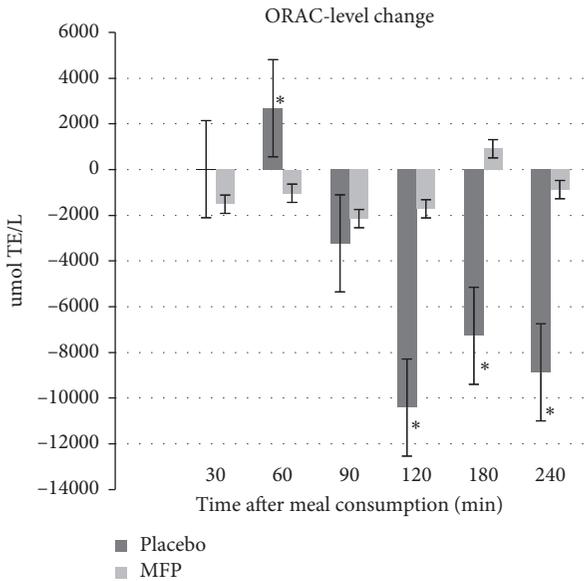


FIGURE 4: Postprandial blood ORAC level change from the beginning (min 0) in MFP jelly and control groups, data expressed as mean \pm SE. *Statistically significant with p value < 0.05 .

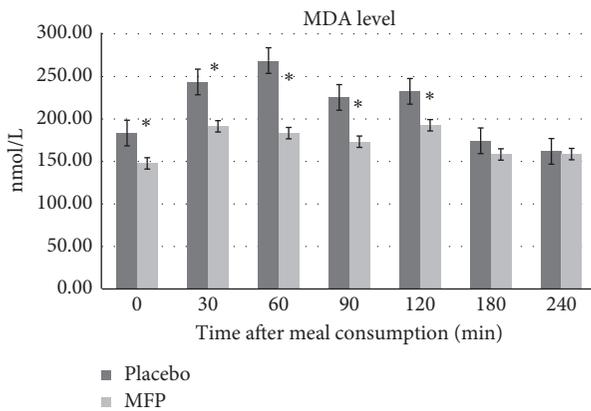


FIGURE 5: Postprandial blood MDA level in the MFP jelly and placebo test, data expressed as mean \pm SE. *Statistically significant with p value < 0.05 .

berry capsule) vs placebo. Anthocyanin supplementation showed significant effects on decreasing serum LDL, TG, glucose, HOMA-IR, apo B-48, apo C-III, IL-6, and TNF- α , while increasing serum HDL ($p < 0.05$) [36]. One possible mechanism of C3G on glucose and insulin responses could be lipoprotein lipase activity. This improves insulin signaling and reduces glucose absorption from the gut [37]. Moreover, C3G lowered postprandial hyperglycemia by GLUT-4 translocation in the soleus muscle by activation of both insulin- and AMPK-signaling pathways [38, 39]. This mechanism might describe the lipid lowering effects of anthocyanins, including reducing blood cholesterol by suppressing the gene expression of hepatic HMG-CoA reductase, resulting in inhibition of cholesterol syntheses,

improved clearance of LDL via increase in the LDL-receptor gene expression and cholesterol excretion in feces [40].

However, variation in the effect of anthocyanin dose might depend on the characteristics of subjects, the intervention period, and observation parameters. Previous studies [14, 34] demonstrated that seven days of repeated anthocyanin intake at 138–600 mg showed a trend to reduce postprandial blood glucose and insulin in various subject characteristics. With more than twelve weeks of intervention period and double the dose of anthocyanin from the present study, effects of anthocyanin berries in triglyceride and HDL improvement were reported [36, 41, 42].

High-fat diets, rich in saturated fatty acid, trigger oxidative stress response by several underlying mechanisms, and consequently, inflammation of endothelial cells causes the initiation of atherosclerosis [43]. This study showed that MFP jelly totally maintained the antioxidant capacities as interpreted by ORAC assays after a high-fat meal intake. Moreover, MDA as an oxidative stress marker induced by a high-fat meal decreased [3, 44, 45]. Therefore, consumption of MFP jelly for seven days and after a high-fat meal might maintain antioxidant capacity by ORAC assay and reduce lipid oxidation by MDA measurement. Several mechanisms have revealed antioxidant activities in anthocyanins including suppressing reactive species formation via inhibitory enzymes, eliminating trace elements related to free radical production, inhibiting lipid peroxidation, and increasing cytokine production, hence regulating immune responses [46].

Therefore, seven days of MFP jelly consumption with 190 mg anthocyanin intake per day was shown to improve blood cardiometabolic risk factors by decreasing cholesterol and LDL cholesterol, and inflammatory markers such as IL-6 in dyslipidemia subjects. Moreover, after seven days of intervention with high-fat meal consumption, postprandial analysis showed that MFP jelly increased insulin sensitivity, reduced oxidative stress, and maintained antioxidant activity of the subjects. MFP jelly contains bioactive compounds and also other nutrients such as fiber, vitamins, and minerals. Thus, MFP jelly offers advantages as a functional food product for dyslipidemia patients.

The limitation in the present study was the study could not be blind because the jelly from mulberry fruit and control was very different. To confirm the effect of MFP on oxidative stress, the further study should investigate other common oxidative stress markers including isoprostanes or oxidized LDL.

5. Conclusions

Anthocyanin-rich mulberry fruit powder (MFP) jelly was successfully developed by adding 14 g MFP with consumer acceptability. This study investigated the effect of short-term consumption of MFP jelly in dyslipidemia subjects. MFP jelly intervention showed potential effects on hypocholesterolemia, insulin sensitivity, and antioxidant capacities. Therefore, acute consumption of MFP jelly could reduce the risk of atherosclerosis and mitigate the development of CVDs and other chronic illnesses.

Data Availability

The chromatogram data used to support the findings of this study are included within the supplementary information file.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Supplementary Materials

Figure 1: high-performance liquid chromatography (HPLC) chromatograms of (A) cyanidin 422 chloride standard, (B) mulberry fruit power (MFP), (C) jelly, and (D) MFP jelly. The retention 423 times (Rt) of cyanidin chloride in the samples were also indicated 424. Figure 2: high-performance liquid chromatography (HPLC) chromatograms of (A) 425 kuromanin and keracyanin standards, (B) mulberry fruit power (MFP), (C) jelly, and (D) MFP 426 jelly. The retention times (Rt) of kuromanin and keracyanin in the samples were also indicated. (*Supplementary Materials*)

References

- [1] C. P. Cannon, "Cardiovascular disease and modifiable cardiometabolic risk factors," *Clinical Cornerstone*, vol. 8, no. 3, pp. 11–28, 2007.
- [2] J.-H. Bae, E. Bassenge, K.-B. Kim et al., "Postprandial hypertriglyceridemia impairs endothelial function by enhanced oxidant stress," *Atherosclerosis*, vol. 155, no. 2, pp. 517–523, 2001.
- [3] S. Tangvarasittichai, "Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus," *World Journal of Diabetes*, vol. 6, no. 3, pp. 456–480, 2015.
- [4] I. Urquiaga, F. Ávila, G. Echeverria, D. Perez, S. Trejo, and F. Leighton, "A Chilean berry concentrate protects against postprandial oxidative stress and increases plasma antioxidant activity in healthy humans," *Oxidative Medicine and Cellular Longevity*, vol. 2017, pp. 1–13, 2017.
- [5] M. Gobert, D. Rémond, M. Loonis, C. Buffière, V. Santé-Lhoutellier, and C. S. Dufour, "Fruits, vegetables and their polyphenols protect dietary lipids from oxidation during gastric digestion," *Food & Function*, vol. 5, no. 9, pp. 2166–2174, 2014.
- [6] Y. Jiang and W.-J. Nie, "Chemical properties in fruits of mulberry species from the Xinjiang province of China," *Food Chemistry*, vol. 174, pp. 460–466, 2015.
- [7] V. R. Kumar and C. S. Mulberry, "Life enhancer," *Journal of Medicinal Plants Research*, vol. 2, no. 10, pp. 271–278, 2008.
- [8] S.-H. Bae and H.-J. Suh, "Antioxidant activities of five different mulberry cultivars in Korea," *LWT—Food Science and Technology*, vol. 40, no. 6, pp. 955–962, 2007.
- [9] K. H. Choi, J.-H. Kang, and J.-S. Han, "Alleviating effects of mulberry fruit extract on postprandial hyperglycemia in streptozotocin-induced diabetic mice," *Journal of Life Science*, vol. 26, no. 8, pp. 921–927, 2016.
- [10] L.-K. Liu, F.-P. Chou, Y.-C. Chen, C.-C. Chyau, H.-H. Ho, and C.-J. Wang, "Effects of mulberry (*Morus alba*L.) extracts on lipid homeostasis in vitro and in vivo," *Journal of Agricultural and Food Chemistry*, vol. 57, no. 16, pp. 7605–7611, 2009.
- [11] M. Polumackanycz, T. Sledzinski, E. Goyke, M. Wesolowski, and A. Viapiana, "A Comparative study on the phenolic composition and biological activities of *Morus alba* L. commercial samples," *Molecules*, vol. 24, no. 17, p. 3082, 2019.
- [12] N. M. Hassimotto, V. Moreira, N. G. do Nascimento, P. C. Souto, C. Teixeira, and F. M. Lajolo, "Inhibition of carrageenan-induced acute inflammation in mice by oral administration of anthocyanin mixture from wild mulberry and cyanidin-3- glucoside," *BioMed Research International*, vol. 2013, Article ID 146716, 10 pages, 2013.
- [13] M. L. Castro-Acosta, L. Smith, R. J. Miller, D. I. McCarthy, J. A. Farrimond, and W. L. Hall, "Drinks containing anthocyanin-rich blackcurrant extract decrease postprandial blood glucose, insulin and incretin concentrations," *The Journal of Nutritional Biochemistry*, vol. 38, pp. 154–161, 2016.
- [14] M. E. T. Willems, J. D. S. Silva, M. D. Cook, and S. D. Blacker, "Beneficial effects on fasting insulin and postprandial responses through 7-day intake of New Zealand blackcurrant powder," *Functional Foods in Health and Disease*, vol. 7, no. 7, pp. 483–493, 2017.
- [15] G. W. Latimer, *Official Methods of Analysis of AOAC International*, AOAC International, Rockville, MD, USA, 20th edition, 2016.
- [16] E. A. Ainsworth and K. M. Gillespie, "Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent," *Nature Protocols*, vol. 2, no. 4, pp. 875–877, 2007.
- [17] L. R. Fukumoto and G. Mazza, "Assesing antioxidant and prooxidant activities of phenolic compounds," *Journal of Agricultural and Food Chemistry*, vol. 48, no. 8, pp. 3579–3604, 2000.
- [18] K. Thuphairo, P. Sornchan, and U. Suttisansanee, "Bioactive compounds, antioxidant activity and inhibition of key enzymes relevant to Alzheimer's disease from sweet pepper (*Capsicum annuum*) extracts," *Preventive Nutrition and Food Science*, vol. 24, no. 3, pp. 327–337, 2019.
- [19] B. Ou, M. Hampsch-Woodill, and R. L. Prior, "Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe," *Journal of Agricultural and Food Chemistry*, vol. 49, no. 10, pp. 4619–4626, 2001.
- [20] I. F. F. Benzie and J. J. Strain, "The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay," *Analytical Biochemistry*, vol. 239, no. 1, pp. 70–76, 1996.
- [21] U. Suttisansanee, S. Charoenkiatkul, B. Jongruaysup, S. Tabtimsri, D. Siriwan, and P. Temviriyankul, "Mulberry fruit cultivar 'chiang mai' prevents beta-amyloid toxicity in PC12 neuronal cells and in a *Drosophila* model of Alzheimer's disease," *Molecules*, vol. 25, no. 8, p. 1837, 2020.
- [22] M. Meilgaard, G. V. Civille, and B. T. Carr, *Sensory Evaluation Techniques*, CRC Press, Boca Raton, FL, USA, 1999.
- [23] Y. Bhutia, A. Ghosh, M. L. Sherpa, R. Pal, and P. K. Mohanta, "Serum malondialdehyde level: surrogate stress marker in the Sikkimese diabetics," *Journal of Natural Science, Biology, and Medicine*, vol. 2, no. 1, pp. 107–112, 2011.

- [24] M. Sotoyama, S. Uchida, S. Tanaka et al., "Citric acid suppresses the bitter taste of olopatadine hydrochloride orally disintegrating tablets," *Biological & Pharmaceutical Bulletin*, vol. 40, no. 4, pp. 451–457, 2017.
- [25] J. Ventura, F. Alarcón-Aguilar, R. Roman-Ramos et al., "Quality and antioxidant properties of a reduced-sugar pomegranate juice jelly with an aqueous extract of pomegranate peels," *Food Chemistry*, vol. 136, no. 1, pp. 109–115, 2013.
- [26] J. H. Giese, "Alternative sweeteners and bulking agents," *Food technology*, vol. 47, pp. 113–126, 1993.
- [27] K. J. Wallis, "Sucralose: features and benefits," *Food Australia*, vol. 45, no. 12, pp. 578–580, 1993.
- [28] L. Liang, M. Zhu, F. Li et al., "Chemical composition, nutritional value, and antioxidant activities of eight mulberry cultivars from China," *Pharmacognosy Magazine*, vol. 8, no. 31, pp. 215–224, 2012.
- [29] P. R. Patel, N. B. Gol, and T. V. Ramana Rao, "Physiochemical changes in sunberry (*Physalis minima* L.) fruit during growth and ripening," *Fruits*, vol. 66, no. 1, pp. 37–46, 2011.
- [30] M. M. Özcan and H. Haciseferoğulları, "The Strawberry (*arbutus unedo* L.) fruits: chemical composition, physical properties and mineral contents," *Journal of Food Engineering*, vol. 78, no. 3, pp. 1022–1028, 2007.
- [31] C. Sripum, R. K. Kukreja, S. Charoenkiatkul, W. Kriengsinyos, and U. Suttisansanee, "The effect of extraction conditions on antioxidant activities and total phenolic contents of different processed Thai Jasmine rice," *International Food Research*, vol. 24, no. 4, pp. 1644–1650, 2017.
- [32] E. Delgado-Licon, A. L. M. Ayala, N. E. Rocha-Guzman et al., "Influence of extrusion on the bioactive compounds and the antioxidant capacity of the bean/corn mixtures," *International Journal of Food Sciences and Nutrition*, vol. 60, no. 6, pp. 522–532, 2009.
- [33] E. A. Abd El-Hady and R. A. Habiba, "Effect of soaking and extrusion condition on anti-nutrients and protein digestibility of legume seed," *LWT Food Science and Technology*, vol. 36, no. 3, pp. 285–293, 2003.
- [34] P. Solverson, W. Rumpler, J. Leger et al., "Blackberry feeding increases fat oxidation and improves insulin sensitivity in overweight and obese males," *Nutrients*, vol. 10, no. 8, p. 1048, 2018.
- [35] C. L. Sardo, J. P. Kitzmiller, G. Apseloff et al., "An open-label randomized crossover trial of lyophilized black raspberries on postprandial inflammation in older overweight males," *American Journal of Therapeutics*, vol. 23, no. 1, pp. e86–e91, 2016.
- [36] D. Li, Y. Zhang, Y. Liu, R. Sun, and M. Xia, "Purified anthocyanin supplementation reduces dyslipidemia, enhances antioxidant capacity, and prevents insulin resistance in diabetic patients," *The Journal of Nutrition*, vol. 145, no. 4, pp. 742–748, 2015.
- [37] T. Tsuda, F. Horio, K. Uchida, H. Aoki, and T. Osawa, "Dietary cyanidin 3-O- β -D-glucoside-rich purple corn color prevents obesity and ameliorates hyperglycemia in mice," *The Journal of Nutrition*, vol. 133, no. 7, pp. 2125–2130, 2003.
- [38] J. Yamamoto, A. Naemura, M. Ura et al., "Testing various fruits for anti-thrombotic effect: I. mulberries," *Platelets*, vol. 17, no. 8, pp. 555–564, 2006.
- [39] Y. Yamashita, L. Wang, F. Nanba, C. Ito, T. Toda, and H. Ashida, "Procyanidin promotes translocation of glucose transporter 4 in muscle of mice through activation of insulin and AMPK signaling pathways," *PLoS One*, vol. 11, Article ID e0161704, 2016.
- [40] M. O. de Souza, L. Souza E Silva, C. L. de Brito Magalhães et al., "The hypocholesterolemic activity of açai (*euterpe oleracea* mart.) is mediated by the enhanced expression of the ATP-binding cassette, subfamily G transporters 5 and 8 and low-density lipoprotein receptor genes in the rat," *Nutrition Research*, vol. 32, no. 12, pp. 976–984, 2012.
- [41] A. Basu, N. M. Betts, A. Nguyen, E. D. Newman, D. Fu, and T. J. Lyons, "Freeze-dried strawberries lower serum cholesterol and lipid peroxidation in adults with abdominal adiposity and elevated serum lipids," *The Journal of Nutrition*, vol. 144, no. 6, pp. 830–837, 2014.
- [42] Y. Qin, M. Xia, J. Ma et al., "Anthocyanin supplementation improves serum LDL- and HDL-cholesterol concentrations associated with the inhibition of cholesteryl ester transfer protein in dyslipidemic subjects," *The American Journal of Clinical Nutrition*, vol. 90, no. 3, pp. 485–492, 2009.
- [43] A. D. Peairs, J. W. Rankin, and Y. W. Lee, "Effects of acute ingestion of different fats on oxidative stress and inflammation in overweight and obese adults," *Nutrition Journal*, vol. 10, no. 1, p. 122, 2011.
- [44] F. Yan and X. Zheng, "Anthocyanin-rich mulberry fruit improves insulin resistance and protects hepatocytes against oxidative stress during hyperglycemia by regulating AMPK/ACC/mTOR pathway," *Journal of Functional Foods*, vol. 30, pp. 270–281, 2017.
- [45] S. B. Kesh, D. Sarkar, and K. Manna, "High-fat diet induces oxidative stress and its impact on metabolic syndrome: Review," *Asian Journal of Pharmaceutical and Clinical Research*, vol. 9, no. 1, pp. 47–52, 2016.
- [46] J. F. Reis, V. V. Monteiro, R. de Souza Gomes et al., "Action mechanism and cardiovascular effect of anthocyanins: a systematic review of animal and human studies," *Journal of Translational Medicine*, vol. 14, no. 1, p. 315, 2016.