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Acceptability and Phytochemical Assessment (Antioxidant, Fiber, Glycemic Index, and Vitamin C) of Mar'ke Bilar Healthy Drink as an Alternative to Obesity Prevention

Daya Terima dan Uji Kandungan Fitokimia (Antioksidan, Serat, Indeks Glikemik, dan Vitamin C) pada Minuman Sehat Mar'ke Bilar sebagai Alternatif Pencegahan Obesitas

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ABSTRACT

Background: Obesity is a multifactorial disease that occurs due to excessive storage of fat tissues. The World Health Organization (WHO) states that obesity and overweight are risk factors for the fifth cause of death in the world. A nutritional status monitoring initiated by the Directorate of Community Nutrition of the Indonesian Ministry of Health in 2018 showed that the percentage of the obese adult population aged >18 years was 28.5%, and the 2018 Basic Health Research found 21.8% of the population. Obesity can be prevented by consuming fruits and tubers that are high in fiber, vitamin C, antioxidants, and low glycemic index (GI) value.
Objectives: This study aims to determine the acceptability and phytochemical test of antioxidant, fiber, glycemic index, and vitamin C of Mar'ke Bilar, a healthy drink.
Methods: Experimental research was conducted using a complete randomized design (CRD) consisting of three treatments and two repetitions. Antioxidant, vitamin C, fiber, and GI levels were assessed afterward.
Results: The organoleptic test results show the highest acceptability obtained in formulation 2 consisting of 30 ml passion fruit, 50 ml persimmon, 50 ml purple sweet potato, and 70 ml water. Formulation 2 contained 39.73 mg/ml antioxidant, 4.0 g fiber, 62% GI, and 56.81 mg vitamin C. This means that Mar'ke Bilar can be used as an alternative to obesity prevention.
Conclusions: Formulation 2 is the selected formulation in which the drink is purplish pink, not too thick, watery, refreshingly sweet, and slightly sour with a total antioxidant content of 39.73 mg/ml, 4.0 g of fiber, GI of 62%, and vitamin C of 56.81 mg.

INTRODUCTION

One of malnutrition, a nutritional problem in Indonesia, is overnutrition or obesity. Obesity is a multifactorial disease that occurs due to excessive storage of fat tissues in adipose tissue. Data showed an increasing trend in the incidence of obesity in adults from 10.5% in 2007, 14.8% in 2013, to 21.8% in 2018[1]. Nutritional status measurements initiated by the Directorate of Public Nutrition of the Indonesian Ministry of Health in 2018 were based on the Body Mass Index (BMI) of adults aged >18 years. The measurement results showed 14.6% of people were overweight, and 28.5% were obese[2]. The World Health Organization (WHO) states that overweight and obesity are the fifth causes of death in the world[3].

The incidence of obesity is influenced by various factors including low physical activity (sedentary life), heredity, westernized eating patterns (consumption of fast food), and low fiber intake due to unmet consumption of vegetables and fruit[4]. Obesity can be overcome through diet, but this action requires high awareness and discipline to keep up healthy eating habits[5]. One example of healthy eating habits is consuming alternative food containing fiber, high antioxidants, and low glycemic index (GI) and avoiding high-fat food. Fruit and tubers (potatoes, sweet potatoes, cassava) are food that has a relatively lower GI[6,7].

Utilizing local food sources, including fruit and tubers in North Sumatra, can be used as an alternative to overcome obesity. Purple passion fruit and persimmon are often found in the highlands of North Sumatra, especially Berastagi. Purple passion fruit, persimmon, and purple sweet potato can be combined to produce a healthy drink known as Mar'ke Bilar. The development of

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diversified food products, especially those made from local typical ingredients from North Sumatra, has received great attention in recent years. Apart from introducing typical products in North Sumatra, such local food contains natural antioxidant bio-active substances that are needed to prevent and overcome various non-communicable diseases[7,8].

Natural exogenous antioxidants are found in food that contain vitamins and minerals such as fruit, vegetables, nuts, and tubers. Antioxidants contained in the food are bioactive substances such as anthocyanins, beta-carotene, and flavonoids (phenols, tannins which are phytochemical substances needed to maintain and improve health)[9]. The formulation of the Mar'ke Bilar which comes from purple sweet potatoes has a sweet taste, and 100 grams of ingredients contain anthocyanin (61.85) mg, and zinc mineral (0.27 – 1.89 mg). This purple sweet potato can also be used as a sugar substitute, thickener, and natural coloring[10]. Purple passion fruit is a typical fruit from North Sumatra that has a distinctive citrus aroma and high vitamin C content, and 100 grams of the fruit contains 88 mg of vitamin C[11,12]. Purple passion fruit also contains high fiber which can be used as an alternative for obesity prevention. Purple passion fruit is a fruit typical in North Sumatra which has a citrus aroma. While persimmons are rich in beta-carotene and bioactive substances in the form of phenols and tannins, 100 grams of persimmons contains 109 mcg of beta-carotene and 11 mg of vitamin C[13,14]. In previous studies, the ingredients for making the Mar'ke Bilar drink, such as purple passion fruit, persimmons, and purple sweet potatoes, have never been combined in one concoction for obesity prevention. Passion fruit and persimmons are used to sweeten the drink, while purple sweet potatoes are used to thicken it. Thus, the drink does not use any granulated sugar as a sweetener. The idea above underlies the aim of the research to determine the acceptability and the phytochemical test of antioxidants, fiber, glycemic index, and vitamin C levels contained in Mar'ke Bilar to prevent obesity.

METHODS

This study was experimental research using a completely randomized design (CRD) with three treatments and two repetitions of the treatments. An organoleptic test of Mar'ke Bilar was carried out at the Food Technology Laboratory, Nutrition Department, Medan Health Polytechnic, while the phytochemical test was carried out at the FMIPA Chemistry and THP (Agricultural Products Technology) laboratory, Brawijaya University, Malang. This study started from 3 to 18 April 2023 using three healthy drink formulations: purple sweet potato juice (40:50:60 ml), persimmon juice (40:50:60 ml), purple passion fruit juice (30:30:30 ml), and water (90:70:50 ml) (see Table 1). Then, to determine the acceptability of the Mar'ke Bilar drink, an organoleptic test was carried out using 50 panelists. The research was approved by the ethics committee from the State Health Polytechnique of Medan, No: 01.1500/KEPK/POLTEKKES KEMENKES MEDAN 2023.

Tools and materials

In making Mar'ke Bilar, the researchers used tools available at the Food Technology Laboratory, Nutrition Department, State Health Polytechnique of Medan, Ministry of Health, Medan. The tools used include blenders, knives, cutting boards, basins, filters, pans, gas stoves, food scales, packaging bottles, and tablespoons. Meanwhile, the ingredients purchased in a local market and used in making Mar'ke Bilar included purple passion fruit, persimmons, and purple sweet potatoes.

Research Stages

Mar'ke Bilar making process

In making the drink, tools, and materials were prepared first, and then the ingredients were weighed with 600 grams of purple sweet potatoes, 600 grams of persimmon, and 600 grams of passion fruit. Next, the purple sweet potatoes were steamed. After steaming, the potatoes' weight was 600 grams. The peeled potatoes had a net weight of 511 grams; the persimmon's weight was 432 grams, and the passion fruit's weight was 294 grams. All three ingredients were blended one by one by adding 86 ml of purple sweet potato, 112 ml of persimmon, and 90 ml of passion fruit. After the mixture was filtered, and it yielded 330 ml of purple sweet potato juice, 330 ml of persimmon, and 180 ml of passion fruit. The drink was made by first mixing purple sweet potatoes and persimmons depending on the three formulas: 40 ml purple sweet potatoes and 40 ml persimmons; 50 ml purple sweet potatoes and 50 ml persimmons; and 60 ml purple sweet potatoes and persimmon 60 ml. A mixture of purple sweet potato juice and persimmon juice was heated with the addition of 90 ml, 70 ml, and 50 ml water. It was then cooled to a lukewarm temperature of 36°C measured using a thermometer). Passion fruit juice was added with these respective ratios: 30 ml, 30 ml, and 30 ml. Next, all juices were packaged into 200 ml bottles.

Table 1. Distribution of ingredients in making Mar'ke Bilar

Materials	Unit	Formulation			Total
		F1	F2	F3	
Purple sweet potato juice	ml	40	50	60	150
Persimmon juice	ml	40	50	60	150
Purple passion fruit essence	ml	30	30	30	90
Water	ml	90	70	50	210

ml = milliliters

Antioxidant testing using the diphenylpicrylhydrazyl (DPPH) method

For the content testing, 0.2 mL sample at various concentrations was pipetted with a micropipette and put into a vial, then added with 3.8 mL of 50 µM DPPH solution. The mixture was then shaken until homogeneous and left for 30 minutes in a dark place. It was then measured using UV-Vis spectrophotometry (Ultra Violet and Visible) at the

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maximum wavelength of DPPH11. The antioxidant activity of the samples based on the amount of DPPH. The radical absorption inhibition can be determined by calculating the percentage of DPPH absorption inhibition using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Abs. Blanko} - \text{Abs. Sample}}{\text{Abs. Blanko}} \text{x} 100\%$$

Information:
Abs. Blank = absorbance of 50 µM DPPH
Abs. Sample = absorbance of the test samples

Fiber testing

Fiber testing was employed using a starch and protein hydrolysis method with the principles of the gravimetric enzymatics. Residue filtration separated insoluble and non-hydrolyzable molecules. In this process, the fiber residue was first dried and then weighed. The residue was then analyzed for protein and ash content. Food fiber content was obtained after the residue was reduced by protein content and ash content (AOAC Official Method 991.43, 2000)[4].

$$\text{Fiber (\%)} = \frac{\text{W2} - \text{W1}}{\text{W}} \text{x} 100\%$$

Information:
W1 = weight of empty filter paper (gram)
W2 = weight of filter paper and residue after oven (gram)
W = sample weight (gram)

Glycemic Index (IG) Testing

The glycemic index test was carried out on mice before they fasted for 10 hours at night. Afterward, their blood glucose levels were checked. Rats were still given normal rations before fasting, and they did not do strenuous activities. Blood glucose was measured in 10 mice that had previously consumed standard food (pure glucose), and the next morning blood samples were taken in the capillaries at minute 0, 30, 60, 90, and 120.

The trial of the Mar'ke Bilar on mice, which had consumed standard food and were given 250-500 mL of water, was carried out on the fourth day. Blood sugar levels (at each time of sampling) were plotted on two axes, namely the time (X) and blood sugar levels (Y). GI determination was carried out by comparing the area under the curve between the test food and the reference food (pure glucose) multiplied by 100. The Brouns et al.'s (2005) method was used as a reference for calculating the area under the curve with the following formula:

$$\text{L} = \frac{\Delta 30\text{t}}{2} + \Delta 60\text{t} + \frac{(\Delta 30 - \Delta 60)\text{t}}{2} + \Delta 90\text{t} + \frac{(\Delta 60 - \Delta 90)\text{t}}{2} + \Delta 120\text{t} + \frac{(\Delta 90 - \Delta 120)\text{t}}{2}$$

Information:
L = Area under the curve
t = Blood collection time interval within 30 minutes
Δ30 = difference in blood glucose levels within 30 minutes after load and fasting
Δ60 = difference in blood glucose levels within 60 minutes after load and fasting
Δ90 = difference in blood glucose levels within 90 minutes after load and fasting
Δ120 = difference in blood glucose levels within 120 minutes after load and fasting

The GI calculation was carried out by comparing the curve area of the test food sample (Mar'ke Bilar) and the curve area of the reference food (pure glucose). The following is a formula for determining the glycemic index of food[15].

$$\text{IG} = \frac{\text{Area of samples'curve}}{\text{Standard area of curve (pure glucose)}} \text{x } 100$$

Testing vitamin C levels using the iodometric titration method

A total of 10 ml of each sample was put into a measuring flask with a capacity of 100 ml, diluted using distilled water until the mark; then, 10 ml of filtrate for each sample was pipetted and put into a 250 ml Erlenmeyer flask, added with 2 ml of 1% starch solution and, if possible, added with 20 ml of distilled water. Next, 0.01 N I2 solution as the titrate was used until the samples changed color to blue.

$$\text{Vitamin C levels (\%)} = \frac{\text{100 x mg ascorbic acid x fp}}{\text{mg sample}}$$

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Information:
Fp = dilution factor
1 mg 0.01 N Iodine = 0.88 mg ascorbic acid
mg ascorbic acid = 0.88 xsample titration volume

Data Analysis

The Mar'ke bilar drink was tested organoleptically on a hedonic scale to determine the formulation's acceptability. It was then analyzed using analysis of variance (ANOVA) followed by the Duncan test. From the organoleptic results, one formulation was then determined to be tested for phytochemical content (antioxidants, fiber, GI and vitamin C). Phytochemical testing with the selected formulation was carried out at the THP (Agricultural Products Technology) Laboratory and Chemistry FMIPA, Brawijaya University, Malang, and then analyzed descriptively.

RESULTS AND DISCUSSION

Organoleptic Test Results

Organoleptic test results were presented using a hedonic scale according to various formulations in which purple passion fruit, persimmon, and purple sweet potato play as thickeners and sweeteners. The organoleptic test domains assessed include color, texture, taste, and aroma as well as overall test[16]. Table 2 shows the distribution of organoleptic results of the Mar'ke Bilar.

Table 2. Distribution of organoleptic test results based on various ingredient formulations

Organoleptic Properties	Treatment			p-value
	F1	F2	F3	
Color	4.01b**	4.16b**	3.76a**	0.001*
Texture	3.74a**	4.24b**	3.5a**	0.000*
Flavor	4.02a**	4.34b**	3.9a**	0.002*
Aroma	3.66a**	4.14b**	3.54a**	0.000*
Average	3.85	4.22	3.67	

ANOVA test; *) Significant if p-value <0.05
DUNCAN test; **) letter notation a = like, b = really like

Table 2 presents the results of organoleptic tests which include color, texture, taste, aroma, and overall acceptability. This study found that formulation 2 was the most preferred among the three formulas tested. Formulation 2 consists of 30 ml purple passion fruit juice, 50 ml persimmon juice, 50 ml purple sweet potato juice, and 70 ml water. The formulation-2 drink was purplish pink, slightly thick, sweet, and clean without granule residues in the mouth. Meanwhile, the aroma of Mar'ke Bilar was very distinctive citric, boosting a consumer's appetite.

Color

Color is the organoleptic domain that gives the first impression and message to the human response (panelists) even when seen from a distance[17]. The organoleptic test results show that formulation 2, the most preferred formula, had 4.16 of color value yielding a purplish-pink color. It had this color because of a combination of orange and dark yellow content produced by the pigment betaxanthin in persimmons, as well as the light-yellow color produced by the pigment beta-carotene in passion fruit. After mixing the color combination with the dark purple color of purple sweet potatoes produced by anthocyanin, the final color was mirabella[17,18]. The bright color produced from yellow and orange pigments remained because the acid from purple passion fruit could extract the anthocyanin pigment in purple sweet potatoes, resulting in a brighter and more attractive mirabella (pH 2.0 – 4.0). The color can be found when the formulation drink was added with 50 ml of purple sweet potato juice, not giving a darker color despite the acidic atmosphere (pH < 2)[19,20]. Apart from the acidic atmosphere brightening the color of the Mar'ke Bilar , it turns out that the steaming process in extracting the purple sweet potato juice could maintain the purple content to be 3.2 times lighter[21].

Texture

Texture is an indicator of an organoleptic test that provides a sensation through observation and smelling using sensorial organs such as the mouth (tongue), index finger, and thumb to touch. The organoleptic test results showed that formulation-2 drink was the most preferred due to creating neither too thick nor watery texture without any residue[4,22]. Such texture might come from carbohydrate content in the form of sucrose in purple sweet potatoes and fructose in persimmons stabilizing water holding capacity to avoid viscosity and result in a soft mixture[23]. The texture or viscosity of the drink was influenced by the carbohydrate content in the form of monosaccharides (glucose and fructose) and fiber after the addition of purple sweet potatoes. This study found that 50 ml of purple sweet potato juice produced a less thick mixture, possibly because processing purple sweet potatoes into purple sweet potato juice was only done through steaming. Therefore, the steaming process did not result in clumping[19].

Flavor

Taste is one of the human sensory responses dominantly used in evaluating a product. The organoleptic test results showed that the formulation-2 drink was the most preferred because it had a sweet yet slightly sour taste but had a fresh taste when drunk[4,24]. The sweet taste arose due to the starch content in purple sweet potatoes and the fructose content in persimmons. The sour taste also provided a fresh taste resulting from the combination of carbohydrate and acid sources with the right

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composition. The refreshing taste of Mar'ke Bilar could also be triggered by the maldoxtrin content in purple sweet potatoes and also the steaming and heating processes which did not use excessive heat. The persimmon juice, for instance, was heated for about five minutes, and the heating process made the sweet taste more natural[19]. The combination of sourness and sweetness influences the sensory response in the hypothalamus which causes a person to like the Mar'ke Bilar which has distinctive color and taste. Therefore, taste is one of the most important factors in food testing because it deals with palatability[25].

Aroma

Aroma is a response that occurs when the volatile compounds of a food ingredient are inhaled into the nose and smelled by the olfactory system[24]. The organoleptic test results demonstrated that the formulation-2 drink had a distinctive citrusy passion fruit aroma, being the most favored[4,14]. The strong citric aroma in the purple passion fruit suppresses the unpleasant smell of purple sweet potatoes and persimmons. The sour aroma disappeared during the high-temperature processing. The purple passion fruit processing does not involve heating, and thus citrus aroma from the volatile components, such as carboxylic acid, butyric acid, acetate acid, and isovaleric acid can be maintained[26,27]. The comfortable aroma can be generated when the starch breaks down into simple carbohydrate molecules that smell like heated sugar. When the aroma mixes with the aroma of purple passion fruit, it will change into a very refreshing drink aroma[23,28].

Whole

Formulation-2 drink was composed of 50 ml purple sweet potato juice, 50 ml persimmon juice, 30 ml purple passion fruit juice, and 70 ml water with an average preference of 4.22. Based on the ANOVA test continued with the Duncan test, the formulation-2 drink was neither too thick nor runny but smooth without any residue. The drink tasted slightly fresh sweet, sour, distinctive citric aroma. However, in terms of color, the formulation-1 and formulation-2 drinks had the purple sweet potatoes reduced resulting in a purplish brighter red.

Phytochemical test

The phytochemical content of the formulation-2 drink was carried out to determine several bioactive contents, nutrients, and antioxidants. The phytochemical examination was repeated twice. Further, Table 3 demonstrates the content distribution of Mar'ke Bilar. In Table 3, overall, Mar'ke Bilar has an average total antioxidant content of 39.73 ppm, 4 mg of fiber, 62% of a glycemic index, and 56.81 mg of vitamin C.

Table 3. Distribution of phytochemical content of Mar'ke Bilar

Parameter	Mar'ke Bilar's formulations			
	Deuteronomy 1	Deuteronomy 2	Average	Unit
Antioxidant	39.77	39.69	39.73	ppm
Fiber	4.21	3.72	4	mg
Glycemic Index	59	63	62	%
Vitamin C	56.90	56.72	56.81	mg

ppm = Parts per Million, Mg = milli gram

Antioxidants for obesity prevention

Mar'ke Bilar contains several bioactive substances as antioxidants. These bioactive substances have anti-obesity properties inhibiting the lipogenic process in which the release of fatty acid synthase, lipoprotein lipase, and acetyl synthetase is impeded. The anthocyanin content as an antioxidant can also increase phosphorylase from protein kinase and acetyl coenzyme A. Active acetyl coenzyme A stimulates the carnitine acyl transferase reaction by increasing fatty acid metabolism in which fatty acid formation does not occur and will automatically reduce fat cells in adipose tissue to prevent obesity. Mar'ke Bilar consists of purple passion fruit, persimmon, and purple sweet potato which generate exogenous antioxidants in the form of vitamin C, beta carotene anthocyanin, and flavonoids reducing lipogenesi[29,30]. The formulation-2 Mar'ke Bilar contains a total of 39.73 ppm of antioxidants classified as having a very strong enzyme activity[28]. For antioxidant activity, IC 50 (Inhibition concentration) was used. The classifications of concentrations include 50 µg/mL (very strong), IC 50-100 µg/ml (strong), IC 50 100-150 µg/ml (medium) and IC 50 151 -200 µg/ml (weak)[31].

Fiber for obesity prevention

Dietary fiber is classified into soluble fiber and insoluble fiber, both of which work together to prevent obesity through the binding of soluble fiber with excessive glucose and fat, as well as the function of insoluble fiber to increase the volume of feces. Simple sugar and fat are immediately excreted along with the feces after the fiber processing[32,33]. Fiber functions can be an inhibitor of the metabolic process by slowing the rate of food intake in the digestive tract and suppressing an enzyme activity which causes simple carbohydrate metabolism to slow down and the response to blood glucose processing reduced[33]. Fiber can also increase feelings of fullness and reduce hunger by suppressing the insulin response. Insulin, if not working optimally, can reduce glucose mobility and energy intake, contributing to controlling body weight and preventing obesity[34]. The fiber content of Mar'ke Bilar was 4 grams/100 ml, and this volume could be effective for obesity prevention. If someone consumes 200 ml of the drink, then the daily fiber intake will be around 20-25% every day[4].

Glycemic Index prevents obesity

The formula-2 Mar'ke Bilar contains a glycemic index including a moderate glycemic index. The results of a phytochemical examination showed that the glycemic index was 62%, grouped into the range of 55% – 70%. Food or drink with a low and medium glycemic index can respond to blood glucose weakly by suppressing blood glucose spikes; hence, carbohydrate reserves in the form

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of glycogen can be prevented. In other words, fat will not be formed in the form of triglycerides in adipose tissue[24]. Mar'ke Bilar with a moderate glycemic index reduces the digestibility of carbohydrates from food that enters the body, resulting in increased blood glucose and a slow insulin response[35]. Factors that influence GI include the level of gelatinization, physical form of food, amylose and amylopectin ratio, dietary fiber content, sucrose sugar content, degree of acidity, fat, and protein, and level of doneness. Research conducted by Iova in 2021 showed that regular high-antioxidant food consumption can reduce blood glucose in hyperglycemic mice[36].

Vitamin C for obesity prevention

The vitamin C contained in Mar'ke Bilar was 56.81 mg obtained from a combination of purple sweet potatoes, persimmons, and purple passion fruit. Vitamin C works by inhibiting glucose absorption, stimulating glucose uptake in peripheral tissues, and regulating an enzymatic activity involved in carbohydrate and fat metabolism pathways. Vitamin C can act similarly to insulin, and the glucose process can be used by cells to avoid glycogenesis[12,32]. The results of the 2022 Pandiangan research showed that giving vitamin C of 750 mg/kg BW for 56 days successfully influenced the leptin hormone, and thus its ability to process food uptake decreased[37], preventing obesity to happen[37]. The anti-obesity effect of exogenous antioxidants such as vitamin C can change the mitogen-activated protein kinase (MPAK) and nuclear factor kaffa Beta (NF-kB) signaling pathways playing a cytoprotective role in obesity pathology. Vitamin C also works by reducing intraperitoneal fat and increasing the action of peroxisome proliferator-activated receptors (PPAR) in adipose tissue[30].

CONCLUSIONS

The results of the organoleptic test on the drink preference found that the formulation-2 drink consisted of 50 ml purple sweet potato juice, 50 ml persimmon juice, 30 ml purple passion fruit juice, and 70 ml water. Based on the ANOVA test continued with the Duncan test, the second formulation, the most favored drink, had some characteristics: a purplish pink color (mirabella), smooth texture, with any residue, sweet and slightly sour taste, and fresh, distinctive citric aroma. Mar'ke Bilar contained a total antioxidant content of 39.73, 4 mg fiber, a glycemic index of 62%, and vitamin C 56.81 mg.

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