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Functional Food Development of Natural Colorant Additives from Mulberry Fruit Extract (*Morus nigra* L) with Bioactive and Hedonic Evaluation

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ABSTRACT

All modern lifestyles such as unhealthy eating patterns and lack of exercise can trigger the accumulation of free radicals in the human body, where the human body cannot neutralize these free radicals fast enough because of the large number of free radicals that have accumulated in it. Therefore, antioxidant compounds need to be consumed to suppress free radicals. In this study, mulberry is promoted as a natural, functional, and dye source that has been explored for further applications in food and beverages. This research aims to develop natural coloring products from mulberry fruit through extraction and encapsulation processes. Furthermore, hedonic testing was also carried out to determine the panelist's preference level for jelly products using mulberry fruit dyes. Mulberry fruit is extracted, encapsulated by freeze drying method, and processed into a natural dye in jelly products. Mulberry fruit extract contains vitamin C 4.1 g/ml, anthocyanin content of 215.16 mg/L, and antioxidant activity of 73.25. Mulberry encapsulates can be used as a natural dye in jelly products. Based on hedonic testing, jelly products made from natural dyes have superior taste and texture. Further development of natural mulberry fruit encapsulated dyes needs to be done to increase solubility, color sharpness, aroma and color stability.

Keywords: *mulberry; functional; encapsulation; hedonic; jelly*

INTRODUCTION

Living in the modern era, where free radicals are becoming a huge threat possessed to human health. Free radicals come basically from normal essential metabolic processes in the human body. However, they are exacerbated by external sources such as exposure to x-rays, ozone, smoking, or industrial chemicals (Cheng *et a*l., 2022). Furthermore, the human lifestyle at this fast pace also contributes to the production of radicals in the body, which makes people buy and consume their food quickly without much consideration (Lee *et al*., 2022). Society tends to select instant foods that are easily accessible and fast, even though people know consciously that these foods contain high trans-fat, additives, and preservative ingredients that can be harmful to the human body (Paula Neto *et al.*, 2017)

Free radicals are defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital, able to donate an electron to or accept an electron from other molecules. They can be very reactive in attacking important macromolecules of the body, including lipids, nucleic acids, and protein which are their major targets, leading to cell damage and homeostatic disruption (Martemucci *et al*., 2022). Besides being influenced by genetics and the environment, bad food habits can be more detrimental to humans, because free radicals accumulate with age throughout the body. Recently, the correlation between the free radical process and aging or diseases has been widely studied (Hajam *et al*., 2022).

The main scavenger for these highly reactive free radicals is antioxidants, as the stable molecule that can reduce oxidative damage in the body by reacting directly with reactive oxygen species (ROS) compounds or carrying out indirect reactions by inhibiting the activity of enzymes that trigger the production of free radicals. Antioxidants will donate electrons to

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free radicals and neutralize them, thereby stopping their reactivity (Martemucci *et al*., 2022) (Elsayed Azab *et al*., 2019). The existence of antioxidant compounds is a valuable thing to support a healthier human life, so it is not surprising that studies on the application of antioxidants as a complement to human nutrition are increasing now, in particular from the pigment plant-based (Mohamad *et al*., 2019).

Of the various types of chemical compounds, anthocyanins are substances that are considered to have the potential to be strong antioxidants. This flavonoid compound has many OH bonds, in which those hydroxyl groups are responsible for combating reactive radical species through the donation of the H atom in a single electron transfer reaction. The health benefits of anthocyanin had been studied for cardiovascular and neurodegenerative diseases (Mattioli *et a*l., 2020). Moreover, the anthocyanin compound has several chemical characteristics that support wide implementation in the context of food: 1) the OH bonds cause the high water solubility properties (Yuniati *et a*l., 2021); 2) the compound shows the color variation for different pH: red to blue color (Roy & Rhim, 2020). Fortunately, this compound is found in a variety of fruits and vegetables, including mulberry, as this study concerns.

Mulberry plants are spreading widely across many countries, especially in Indonesia (Nuratika *et al*., 2020). Mulberries are not only consumed as fruit but are also effective as a natural remedy for sore throat, fever, hypertension, and anemia. Furthermore, mulberries are also used as supplementation ingredients that can prevent heart and kidney damage, strengthen limbs, improve sight, and have anti-aging properties. Anthocyanin extract from mulberries had been studied to scavenge free radicals, low-density lipoprotein (LDL) oxidation, and have beneficial effects on blood and atherosclerosis (Wen *et al.*, 2019). Therefore, the potential of mulberry fruit as an antioxidant can still be explored further, to create healthy processed ingredients.

Unfortunately, mulberry's substantial water content, ranging from 60 to 75% causes it perishable and stimulates its bioactive compound easier to degrade (Ali et al., 2016). Encapsulation with a drying base is considered one effective solution to protect the compounds contained in the mulberry fruit (Indrawati et al., 2017). Commercially, freeze-drying is a suitable technique to maintain pigment resilience in mulberry fruit, anthocyanin, as food additives with functional value (Wahyuningsih *et al.*, 2017)(Nafiunisa *et al*., 2017). Therefore, this research was focused on encapsulating mulberry fruit, preceding by electric juice blender and straining the residue (Yuniati *et al.*, 2021)(Indrawati et al., 2017). Before encapsulation process, the extract were measured their functional value by testing vitamin C, anthocyanin content, and antioxidant activity. Product development was carried out by applying mulberry encapsulates as a natural colorant in jelly. The nutritional content contained in jelly makes many people like to consume it. Meanwhile, after producting encapsulate products, they were evaluated the hedonic rating in simple sensory evaluation of colored jelly products, in order to measure the success of this food additive for further appplication. A simple hedonic test was applied to find out the success and chances of mulberry encapsulation when used as a natural coloring agent.

METHODS

Material

The mulberries used in this research belong to the *Morus nigra* L. species. Species classification was done by comparing the shape of the leaf and fruit physically. The other materials used for extraction and lyophilisation process were mineral water (Aqua,

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Indonesia), sugar (Gulaku, Indonesia), maltodextrin and tween 80 (Yishui Dadi Corn Developing Co. Ltd., China). For laboratory analyisis purposes, DPPH or 1,1-diphenyl-2-picrylhydrazyl (Analytical Grade, Sigma Aldrich), Potassium iodide (KI), Sulfuric acid, and starch indikator were used as reagent.

Tool

The equipments used in this study were blender, homogenizer (T-18 Basic, Ultra-Turax Ika, Germany), magnetic stirrer (Labtech, Germany), freeze dryer (Labconco-Freezone 2.5 L Benchtop, USA), sieve (Retcsch, Germany), 4-digit analytical balance (Shimadzu, Japan), and spectrophotometer UV-visible (Shimadzu, Japan).

Procedures

The procedure carried out in this study consisted of several stages: extraction from mulberry fruit; extract analysis of vitamin C, anthocyanin levels, and antioxidant activity; encapsulation-drying process; and sensory evaluation, including preparation of jelly.

In the first step, 500 g of mulberry fruit was washed and steamed for 2 minutes at 70 °C to remove the sap part stuck from the fruit. Then the fruit was allowed to cool down at room temperature and followed by the extraction process. The extraction process was carried out by grinding the fruit directly with a blender for 2-3 minutes at medium speed. The residue pulp formed was then filtered and further processed.

For extract analysis, the vitamin C content was determined through titration, by taking 1 mL of mulberry fruit extract dissolved in distilled water to a volume of 25 mL in a volumetric flask. This mixture was filtered, and 5 mL was taken again to be diluted up to 25 mL with distilled water. This solution was then transferred to a 250 mL erlenmeyer and 2 mL of 0.5 M sulfuric acid solution was added; 0.5 mL of 1% KI solution and 0.5% starch indicator. This mixture was titrated with 0.1 M KI solution until a stable blue color was obtained; hence vitamin C content in the sample was able to be calculated (Arya et al., 2000). Anthocyanin content was determined using the differential pH method by measuring the difference between pH 1.0 and pH 4.5. Fruit extracts at pH 1.0 and pH 4.5 were measured for absorbance value between two wavelengths (513 nm and 700 nm) through a spectrophotometer UV-Vis, where both pHs indicated the amount of anthocyanin content in the fruit extract (Yuniati et al., 2021). Determination of antioxidant activity was carried out using the DPPH test in a dark room. In this method, DPPH solution which acts as a free radical will react with antioxidant compounds so that DPPH will turn into diphenylpycrilhydrazine. An increase in the amount of diphenylpycrilhydrazine will be indicated by a change in purple to pale yellow which can be detected at a wavelength of 517 nm. (Indrawati et al., 2017).

Encapsulation of mulberry fruit extract was conducted by adding 250 ml of extract with 25 g of sugar, 25 g of maltodextrin, and 2.5 g of tween 80. The mixture was stirred with a magnetic stirrer (400 rpm, room temperature) for 15 minutes and with a homogenizer at room temperature for one minute. After the preparation process was completed, it could be frozen in the refrigerator (freezer) at -80 °C for 24 hours. The lyophilization process with a freeze dryer for 48 hours at -43°C under vacuum pressure can only be carried out after the encapsulates are completely frozen. Since the encapsulation was confirmed to obtain a moisture content below 10%, the resulting product was ground with mortal and equalized the particle size by sieve (Indrawati et al., 2017). The mulberry-encapsulated powder was stored in the refrigerator before being used for sensory tests.

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An organoleptic test aims to determine the extent to which a panelist's preference for encapsulated products when used as a food additive in jelly products, compared to another jelly by synthetic colorants. Plain jelly was mixed with 400 mL of water and 50 g of sugar, stirred, and cooked until it cooked. The liquid form of cooked jellyr was then separated into two parts. Coloring additives will be added after the jelly rather cool.The first part was added with 30 g of mulberry encapsulated powder and the second part was added with 3 drops of synthetic red colorant (food grade). The two parts were then stirred until homogeneous and placed into a box until jelly stiffen. Hedonic testing was carried out by involving 25 (twenty-five) non-trained panelists who had once or more consumed jelly. Panelists were shown 2 (two) different jelly formulations, namely sample A (colorant from mulberry encapsulates) and sample B (synthetic colorants).

RESULT AND DISCUSSIONS

Vitamin C, Anthocyanin, and Antioxidant Activity of Mulberry Extract

The vitamin C content of mulberry extract through the iodometric test obtained a result of 4.1 mg in every 1 mL of extract (comprise of 500 g fruit with 72% water content). Hence, the vitamin C content in our study commensurate to medium ripening stage in literature (Saensouk et al., 2022). In order to determine the anthocyanin content of the concentrate, a pH differential test was carried out by measuring the absorbance difference at pH 1.0 and 4.5. The absorbance was measured between two wavelengths: 513 and 700 nm, where both pHs showed the amount of anthocyanin contained. The anthocyanin level in the mulberry extract is known to be 256.5 mg/L. The antioxidant activity in anthocyanin pigment was carried out by a test with the DPPH method in methanol as a solvent. The mulberry fruit extract has an antioxidant activity of 73.25%, match those reported in previous studies (Kim et al., 2009).

Mulberry Extract Encapsulation: Freeze Drying

The making of encapsulated fruit concentrate was aimed to create a product in powder form since the powder form is more stable and can be stored longer so that its antioxidant properties can be preserved. By looking at the results of the total anthocyanin and antioxidant content of mulberry extract between the two previous options, the raw materials used to produce encapsulated products are mulberry extracts produced from the direct extraction process. The coating materials had been confirmed by the food grade, easily accessible, and affordable. Sugar or sucrose as additional sweetener, maltodextrin, and dextrin as a sweetener to speed up the drying process, and tween 80 as an emulsifier so the texture produced is good and stable. Besides its function as a sweetener, dextrin can be used as a filler material in the colorant thus obtaining pigments with high solubility (Cruz-Molina *et al.*, 2021).

The next step in encapsulation was the drying process which aimed to transform the fruit concentrate emulsion product to dried powder form. Freeze drying is still being studied as a drying method that can preserve the product’s nutrition. In this study, the mulberry extracts are shown in Figure 1.

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Figure 1. Dried granule (left) and powder (right) form of mulberry extracts after freeze drying encapsulation

Mulberry Extract as Food Colorant Additives: Hedonic Sensory Evaluation

The organoleptic test was done by comparing encapsulated anthocyanin from mulberry with the synthetic food colorant, as the common food colorant, commercially sold. The test did not necessarily present the panelists in powder form, but the two materials were further applied to become jelly products (Figure 2). With the same formulation, the jelly products could make panelists more comfortable to analyze the advantages and evaluations matter of mulberry food coloring pigment. The jelly was chosen since this product is highly accessible in Indonesia and needs cold storage room, which suits pigments' characteristic (Pandit *et al.*, 2020). The jelly production from mulberry encapsulates was done by heating the jelly mixture in water beforehand until the mixture boils. After the heater was off, the mulberry encapsulate was added to minimize excess degradation of the anthocyanin pigment.

Figure 2. The food colorant additives from mulberry extract (left) and jelly product as a result of further food processing (right)

The organoleptic testing is based on a hedonic test scheme, linking product ratings based on panelist preferences. The test used 25 panelists, combination of students, lecturers, research assistants from Ma Chung University Malang who were accustomed to consuming jelly products as well as sensory analysis of food colorants based on the experiences of various previous research projects. The judged qualities include solubility, color, scent, color stability, taste, and texture. The score ranges between 1 to 5, with 1 (very poor), 2 (poor), 3 (average), 4 (good), and 5 (very good).

Table 1 displays the organoleptic test result given from panels, an average of the score given. A spider web model also shown for simplify comparison, through Figure 3. Overall, jelly from mullberry has a score of more than 3 (average) for all assessment parameters, meaning that the product is quite acceptable by the panelists and has potential to be accepted in the commercial public. By giving the score in the range of 3.7 to 4.4 of mulberry based jelly, close to the results in the assessment of artificial food coloring based (4.2 to 4.5), indicating that the product has proven its worth after being compared with competitor products.

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Table 1. Organoleptic average score from 25 semi-trained panelists in observing jelly from mulberry colorant (Sample A) and synthetic food colorant (Sample B)

Parameter	Sample A	Sample B
Solubility	3.76	4.32
Color	3.76	4.44
Scent	3.80	4.28
Color Stability	4.16	4.52
Taste	4.44	4.24
Texture	4.40	4.20

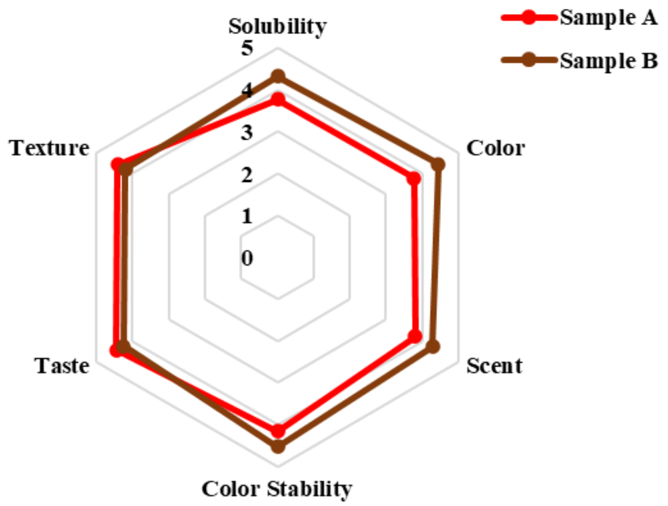

Figure 3. Spider web model of organoleptic test from mulberry colorant jelly (Sample A) and synthetic food colorant jelly (Sample B)

Through this sensory testing, our colorant products can be evaluated. Referring to the score obtained by the panelists, the mulberry jelly is superior in taste and texture, 0.2 up the competitor. It can be assumed that the freeze-drying method helps the preservation of the mulberry color extract in the presence of coating material so that when it is applied as a consumer product, the quality of the ingredients can be maintained from themal processing (Mattioli *et al*., 2020). The better texture of mulberry jelly indicates that the formulation related to the addition of encapsulation material and sugar is as expected by the panelists.

From the results of the sensory test assessment, several things can be used as an evaluation of the mulberry encapsulated product. Previous research related to the use of natural colorant extracts for food product applications claimed that panelists liked the taste of products enriched with natural colorants, so this research supports this previous research (Chaudhary & Singh, 2019). However, the jelly made from synthetic colorants is preferred for solubility, color, aroma, and color stability, Although the score between mulberry jelly and the competitor jelly is not much different. Therefore, the encapsulated product should be improved in the future. Based on the formulation stated in this study, future mulberry encapsulated products are recommended to sharpen their color (in this case increase the mulberry concentration), thereby improving the appearance of the final product.

CONCLUSION

This research succeeded in promoting mulberry fruit as a natural coloring agent for food (the product chosen was jelly in this study). The mulberry extract consists of 4.1 mg/1 ml of

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vitamin C extract, an anthocyanin content of 256.5 mg/L, and an antioxidant activity of 73.25%. Mulberry encapsulation has been successfully applied as a natural colorant for jelly products. The results of simple hedonic testing generally show that natural dyes in the form of mulberry encapsulates still have weaknesses compared to synthetic dyes. The main advantages of natural dyes lie in taste and texture. As for the criteria for solubility, color, aroma, color stability, the value given by the panelists was lower when compared to jelly which was given additional synthetic dyes. The bright purple anthocyanin color is successfully applied in the production of jelly products, and has great potential to balance the quality of jelly products made from non-natural additives. Further research and development still needs to be done, especially to improve the color stability and sharpness before these natural powder food pigments with antioxidant properties can be produced on a large scale and sold widely for consumption.

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