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Effect gelatine of the characteristic functional drink from mangosteen peel extract (*Garcinia Mangostana*)

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Abstract
Anthocyanine had been extracted from mangosteen peel extract and had been formulated to the functional drink of its extract. The formulation was done by adding mangosteen peel extract for about 10%, 20%, 30%, and 40%. The characterization included antioxidant activity, total microbial during storage, organoleptic test, and the stability test Anthocyanine as coloring agent. The formula had antioxidant activity 78% and log total microbe is 2.62 on the second days of storage. Anthocyanine was relative stable in storage temperature used in this research, 5^0C and 25^0C. In this study, the increase in pH will increase the absorbance of anthocyanine, while the presence of hydrogen peroxide leads to absorbance decreases.

Keywords: gelatine, anthocyanine, mangosteen peel, stability, antioxidant

1. Introduction
One of the extract plants that have the opportunity to be developed as a functional drink is mangosteen peel extract. Mangosteen peel extract containing anthocyanine compounds are known to prevent coronary heart disease, stroke, inhibits the growth of tumor cells, antiviral and antimicrobial activity [1]. Anthocyanine also can be used as a natural coloring agent in foods or beverages. The development of mangosteen peel extract is through the creation of functional beverages, namely beverage made with the main ingredient of mangosteen peel extract. To produce the beverage, thus research is needed to determine the right formula in producing good drink and stable during storage.
One ingredient formula used is gelatine. Gelatine is a traslucent, colourless, brittle (when dry), flavourless, foodstuff, derived from collagen obtained from various animal byproducts. Gelatine has viscosity that can affect the solubility of anthocyanine. This research aims to utilize waste mangosteen peel as a functional drink with a way extraction the anthocyanine through different gelatine concentration.

2. Material and Methods
2.1 Plant Material
Samples mangosteen peel (*Garcinia Magostana*) were purchased from traditional market, Bogor, Indonesia.

2.2 Sampel Extraction [2]
Mangosteen peel was cut and washed and then dried using the sunlight. Then extracted using water at a ratio of 1: 8 and filtered to obtain the filtrate. Added the gelatine 0 %, 0.5 %, 1 %, and 1.5 % and precipitated for 12 hours. Furthermore, the filtrate was added ethanol 95 % with a ratio of 2: 1 and filtered. The filtrate was concentrated using the evaporator at a temperature of 50^O C for 3 hours to obtain a thick extract.

2.3 Analysis by Spectrofotometer UV-Vis [3]
Each extract of mangosteen peel weighed 1 g and washed-dissolved in a solution of 10 ml ethanolic-HCl (85:15). The residue sample rinsed with 15 ml of ethanolic-HCl (85: 15), then covered with black plastic and stored in a refrigerator at a temperature 40 C for one night.
And then the sample was filtered with Whatman paper No. 1 into the flask volume 50 ml. The residue on the filter paper rinsed with ethanolic-HCl. A total of 5 ml pipetted into a 50 ml volumetric flask and homogenized with ethanolic-HCl (85: 15), and then the absorbance was measured using visible spectrophotometer at a wavelength of 535 nm.

Anthocyanin levels (mg/100g)

$$= \frac{A \times EC \times FP \times 100}{\text{Weight of sample (g)}}$$

Note: A =Absorbance
EC = Extension Coeficien (98,2)
FP = Dilution Factor

2.4 Antioxidant activity [4]

Antioxidant activity was measured by Radical Scavenger Method. Each of the sample was pipetted 5 ml into 100 ml flask and then diluted using 95 % ethanol. Then homogenized to obtain 5 % solution. Afterwards, the solution was filtered using filter paper to obtain aclear solution purple. Each sample 0.1 ml added DPPH (400 Mikrometer in ethanol) and three ml 95 % ethanol. After that, the mixture was vortexred and measured the absorbance at 0, 5, 10, 15, 20 minutes using UV-Vis at 517 nm [5]

$$\% \text{ Scavenger} = \frac{(A0 - At)}{N0} \times 100\ \%$$

Where
A0 = Absorbance of DPPh solution
At = Absorbance of test reference sample

2.5 Total Microbial During Storage [6]

One ml sample put in a test tube containing 9 ml of diluent (Ringer's solution), whipped and record as a 10^{-1} dilution. From this dilution, pipetted 1 mlinto a test tube containing 9 ml of sterile diluent, vortexes until homogenous and record as a 10^{-2} dilution. The same methods was done to 10^{-3} dilution (for day 0), 10^{-5} (for day 5th), and 10^{-6} (for day 10th).
Then 1 ml sample put into each of sterile petri dish containing 12-15 ml of PCA (Plate Count Agar) at 40 – 45 C then homogenized. The blank with PCA was made too. After freezed, incubated in an incubator at 37 C in the inverted position for 2 X 24 hours. The number of microbes calculated using colony counter and expressed as total colony/ml sample.

2.6 Organoleptic Test [7]

The organoleptic test was done by giving the spesicif code using 3 random numbers. Panelist given assesment form in term of taste. Colour and aroma by giving value to the range of 1-7, which is (very strongly dislike), 2 (strongly dislike), 3 (do not like), 4 (neutral), 5 (like), 6 (very like), 7 (extremely like).

2.7 Stability test of Anthocyanin as coloring agent [8]

2.7.1 Stability test of temperature

The drink mangosteen peel extract stored at room temperature (25 °C) and cold temperature (5 °C), after 2 days measured the absorbance at 535 nm wavelength.

2.7.2 Stability test of pH

Stability drink mangosteen peel extract made in 3 levels of acidity (pH: 3, 4, 5). Two mL dissolved in 100 mL of acetic acid buffer in accordance with the variation of pH. Then measured the absorbance at a wavelength of 535 nm.

2.7.3 Stability test of oxidation H_2O_2

10 mL of mangosteen peel extract drink put in a test tube and added 1 mL oxidant H_2O_2 and then every 3 hours absorbance measurement at a wavelength of 535 nm.

3. Result and Discussion

3.1 The level of anthocyanin

Table 1: The result of the analysis Anthocyanin

Concentration of Gelatine	Level of Anthocyanin
0 %	332,00 mg/100 g
0,5%	389,63 mg/100 g
1%	604,61 mg/100 g
1,5%	409,98 mg/100 g

The addition of gelatine are known to increase the level of tanin deposited thus when the filtration process, the tanin do not get along with the filtrate to be axtracted and matrix or compound impurities is reduced. The lower concentration gelatine and other purification material will act as a protective colloid, otherwise at the higher concentration cannot cause the precipitation again [9]. Based on this, the other characterization used 1 % gelatine.

3.2 Scavenging DPPH of the mangosteen peel extraxt

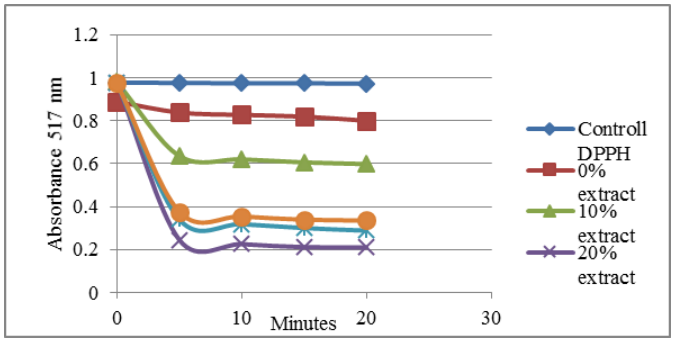

Fig 1: Scavenging DPPH with 1 % gelatine

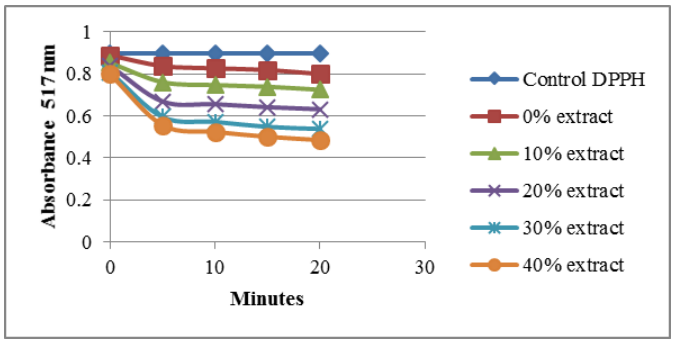

Fig 2: Scavenging DPPH with no gelatine

Table 2: The percentage of scavenging DPPH

No	Mangosteen peel extract	Addition of Gelatine	% Scavenging DPPH
1	0 %	0%	10,01
2	10%	0%	15,00
3	20 %	0%	24,67
4	30%	0%	33,50
5	40%	0%	39,30
6	10%	1%	38,91
7	20%	1%	78,50
8	30%	1%	70,49
9	40%	1%	65,65

*) the tested drink dilluted 20 X

The drink mangosteen peel extract with the addition 1 % gelatine has higher antioxidant activity than without the addition of gelatine [10]. This shows that gelatine has active role increasing the level and the activity of anthocyanin as an

antioxidant. From table 2, it can be known that the higher concentration mangosteen peel extract, then the higher the acitivity antioxidant. The mangosteen peel extract rich antocyanin and xanthone which has the higher antioxidant [11]. The antioxidant in the peel of mangosteen has more than 8 times than the antioxidant in oranges and another fruit such as durian and rambutan.

3.3 Total Microbes During Storage

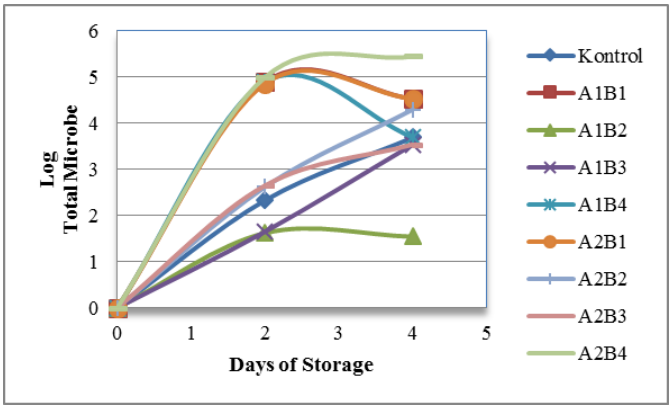

Fig 3: Log total microbes during 4 days of storage

Where:
Control = 0 % extract
A1B1 = 10 % extract A2B1 = 10 % extract + 1 % gelatine
A1B2 = 20 % extract A2B2 = 20 % extract + 1 % gelatine
A1B3 = 30 % extract A2B3 = 30 % extract + 1 % gelatine
A1B4 = 40 % extract A2B4 = 40 % extract + 1 % gelatine

The addition of gelatine has two different function, are as a source nutrient for spesific microbes and also can be used as an antimicrobe [12]. As a nutrition, gelatine is a source of protein which can be used for certain microbes. As a source of incomplete protein (without triptophan), gelatine also has peptide chain that can be penetrate into the membrane of bactery thus inhibits the growth of microbes [2].

3.4 Organoleptic Test

Table 3: Score of the organoleptic test

Formula	Taste Score	Flavour Score	Colour score
Control	5.52	5	4.52
A1B1	5.24	5.04	4.76
A1B2	4.96	4.8	4.72
A1B3	4.52	4.4	4.52
A1B4	3.68	4.04	4.28
A2B1	4.16	4	3.6
A2B2	3.68	3.48	3.6
A2B3	3.16	3.12	3.68
A2B4	2.76	3	3.56

Where:
A1B1 = 10 % extract A2B1 = 10 % extract + 1 % gelatine
A1B2 = 20 % extract 20 % A2B2 = 20 % extract + 1 % gelatine
A1B3 = 30 % extract 30 % A2B3 = 30 % extract + 1 % gelatine
A1B4 = 40 % extract 40 % A2B4 = 40 % ectract + 1 % gelatine

The addition of gelatin will cause the drink becomes more cloudy, smelling like starch and becomes more slimy thus affecting the taste on the tongue. This makes the reduction in assessment score of taste, flavour and color to the drink mangosteen peel extract.

3.5 Stability test of Anthocyanin as coloring agent

1. Stability test of temperature [14]

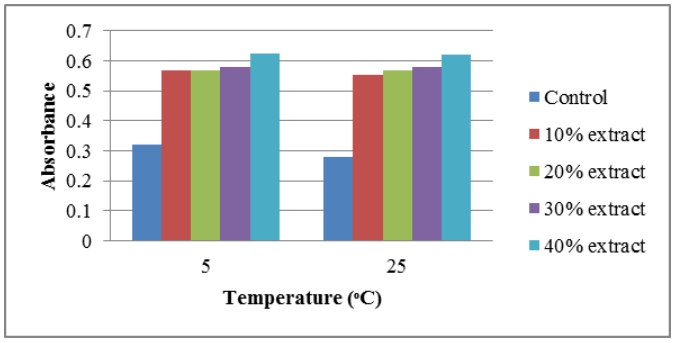

Fig 4: Effect temperature with no gelatin

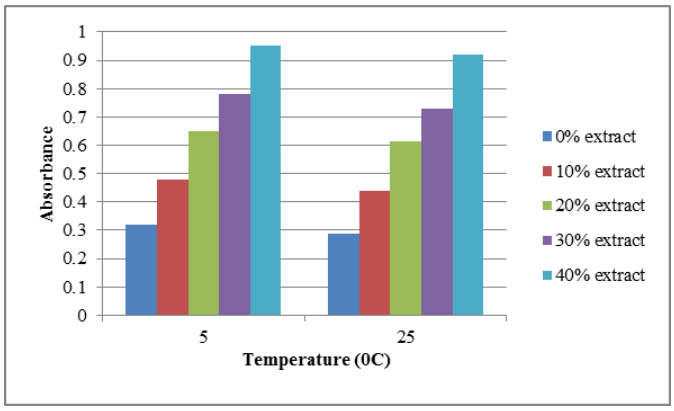

Fig 5: Effect temperature with 1 % gelatine

2. Stability test of pH [15]

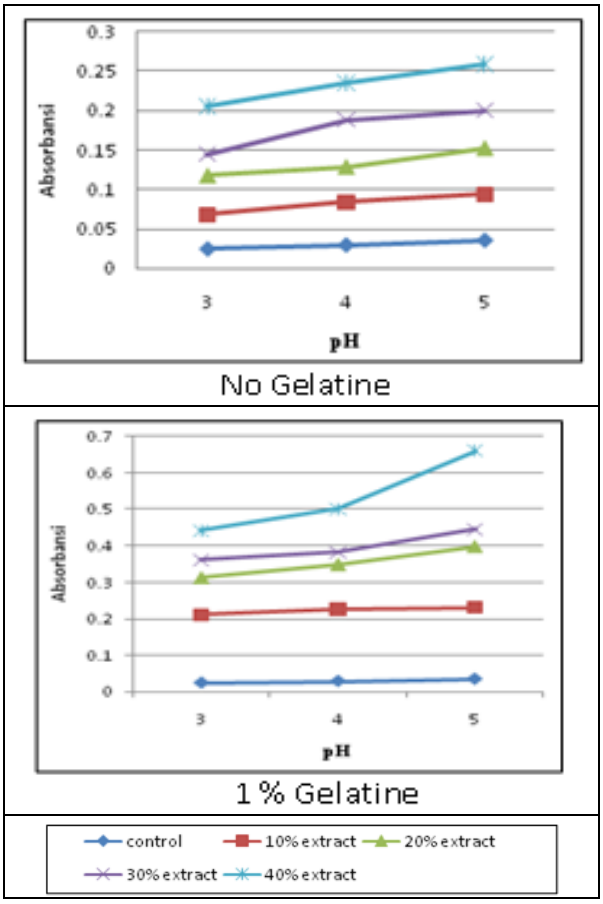

Fig 6: Effect pH of Anthocyanin stability as coloring agent

3. Stability test of oxidator H_2O_2 [16]

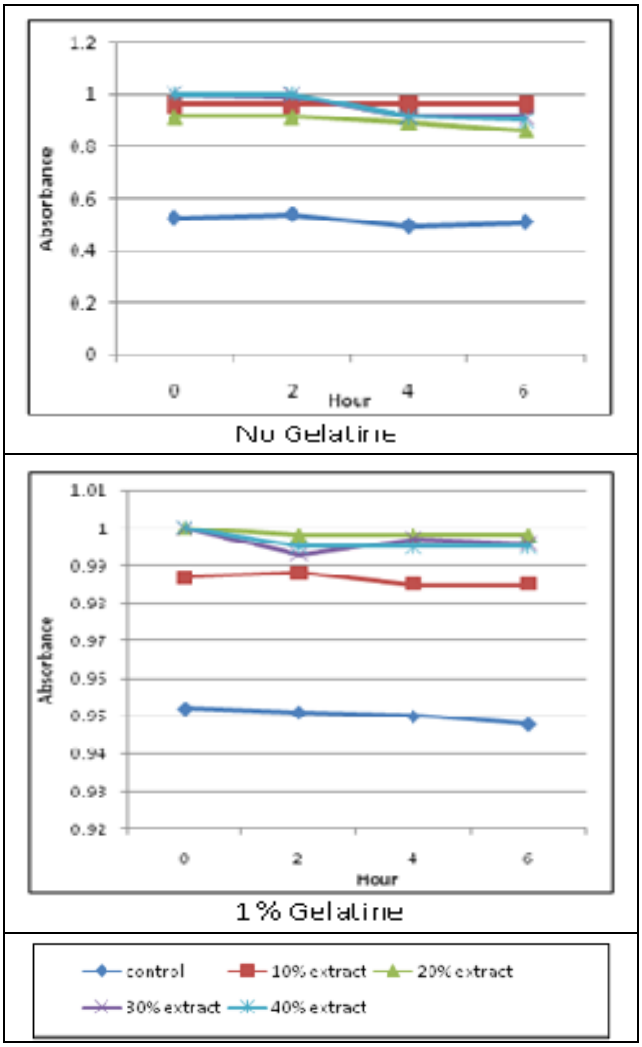

Fig 7: Effect Oxidation H_2O_2 at Anthocyanin stability

Color stability drink mangosteen peel extract is influenced by temperature, pH and the presence of an oxidant. However, in storage at cold temperatures (5 °C) and room temperature (25 °C), the color tends to stable. The increasing of pH makes the color of absorbance higher. Whereas during storage 6 hours oxidation by H_2O_2 thus the absorbance was decreasing [15].

4. Conclusion

The best formulation of mangosteen peel extract is 20 % extract and the addition of gelatine 1 %, because it has the highest antioxidant activity (DPPH reduction to 78.50%), and log total microbial quite low at 2.62 on the 2nd day of storage and relatively slow growth until 4th day. The bigger extract concentration, the lower of panelist's assessment and evaluation will be lower when adding gelatine. Stability test Anthocyanine at the cold storage (5°C) and room temperature (25°C) indicates the color is stable. The increasing of pH (wavelength 535 nm), the color absorbance is higher too. Whereas during storage for 6 hours, oxidation by H_2O_2 made absorbance decreasing.

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