

CHAPTER 3 - RESEARCH METHODS

3.1 Research Design

The study was conducted on the development and evaluation of *Clitoria ternatea* beverage using quantitative and qualitative approaches. In the Focus Group Discussion (FGD) the qualitative approach was used to standardise the taste of the drink ingredients. For laboratory tests of the flower blends and sensory evaluation, a quantitative approach was used to collect numerical scores from consumers. The complete step-by-step workflow for this research design is described below in Figure 3.1.



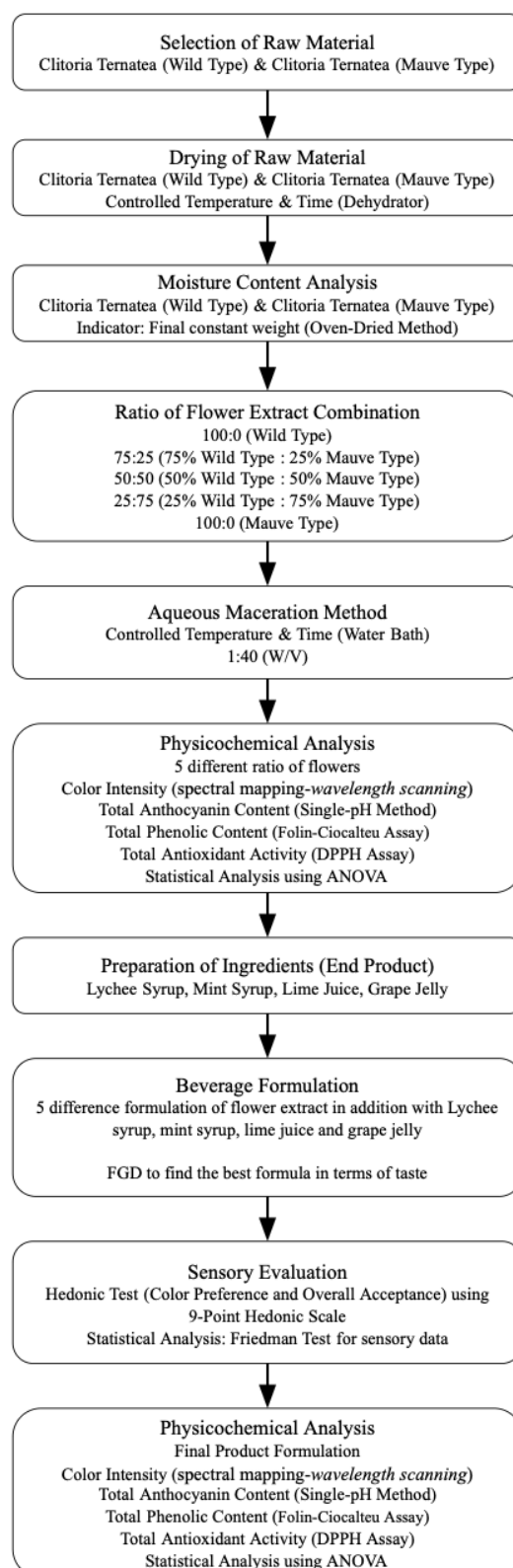


Figure 3.1 Process Flow Diagram of Experiments

In this study, the Wild Type (blue) flower was first mixed with the Mauve mutant (purple) *Clitoria ternatea* flower in five different ratio combinations. The five raw blends were analysed immediately in the laboratory for their physicochemical properties such as color intensity, total anthocyanin content, total phenolic content, and antioxidant activity.

Then, five test recipes for the ingredients of the beverage were tested by Focus Group Discussion (FGD) to determine the base formula. From this, five final drink samples were developed using varying ratios of flower blends. These samples were assessed for consumer color preference and overall acceptance.

Finally, the most preferred sample with the highest consumer scores was further tested for the analysis of its physicochemical and antioxidant properties.

3.2 Data Collection Methods

The data collection for this study was performed in three different phases, beginning with the raw flower extracts, consumer testing and the finished beverage analysis.

In the first phase, flowers of *Clitoria ternatea* (Wild Type and Mauve mutant) were collected, extracted using water and blended in five different ratios. Color and chemical properties of these raw blends were measured by using a UV-Vis spectrophotometer and all lab measurements were performed in triplicate. The step involved the measurement of the peak absorbance and its wavelength, the Total Anthocyanin Content (TAC), the Total Phenolic Content (TPC) and the antioxidant activity using the DPPH radical inhibition assay, following established spectrophotometric screening protocols.

In the second phase of data collection, consumer metrics were obtained using human sensory evaluation. Eighty (n=80) untrained consumer participants were targeted to provide hedonic response profiles using a structured 9-Point Hedonic Scale questionnaire based on standard affective testing methodologies (Meilgaard et al., 2006). Flavour was kept the same for all the samples, after the focus group discussion. Hence, data collection was specific on measuring the preference metrics against two

principal sensory attributes i.e. Color Preference Scores and Overall Acceptance Scores (Meilgaard et al., 2006).

Finally, the data collection was finished with the laboratory analysis of the consumer preferred prototype. The final beverage formulation was filtered to remove solid particles from the liquid and the final chemical values were also determined in triplicate to check analytical accuracy. In this step, the final color intensity, final adjusted TAC, final composite TPC and aggregate functional Total Antioxidant Activity (TAA) were recorded. This three phase process allowed for the clear tracking and measurement of all physical and chemical changes from the raw flower extracts through to the finished consumer product.

3.3 Materials and Equipment

Table 3.1 Materials used in the study

Materials	Formula	Source	Country	Molecular Mass (g/mol)	Purity/Conc. (%)
Butterfly Pea Flower (Wild Type & Mauve Type)	-	Rumah Sains Ilma Pamulang	Indonesia	-	Freshly harvested
Distilled Water	dH_2O	Internal Laboratory Supply	Indonesia		Analytical Grade
Potassium Chloride	KCl	Brand: MerckEMSUR E® Series Number: 1.04936.0500	Germany	74.55	≥99.5

Hydrochloric Acid	HCl	Brand: PT. SMART LAB INDONESIA Series Number: A-1050	Indonesia	36.46	37
DPPH Powder	$C_{18}H_{12}N_5O_5$	Brand: Sigma Aldrich Series Number: D9132-1G	United States	394.32	$\geq 95.0\%$
Methanol (Technical)	CH_3OH	Lab Grade	Indonesia	32.04	$\geq 98.5\%$
Folin-Ciocalteu Reagent	C_6H_6O	Brand: Merck Series Number: 1.09001.0500	Germany	N/A (Liquid Mixture)	2.00 N (Normality)
Gallic Acid	$C_7H_6O_5$	Brand: Sigma Aldrich Series Number: G7384-100G	United States	170.12 (Anhydrous)	$\geq 98.0\%$
Sodium Carbonate	Na_2CO_3	Brand: Merck Series Number: 1.06392.1000	Germany	105.99	$\geq 99.5\%$

Table 3.2 Equipment used in the study

Equipment	Brand	Series Number	Country
Food Dehydrator	LocknLock	EJO316	South Korea
Analytical Balance	Ohaus Pioneer	PX124	United States
Thermostatic Oven	Memmert	B2232307	Germany
Digital Water Bath	Memmert	WNB14	Germany

UV-Vis Spectrophotometer	AELAB	AE-S60 U2	China
pH Meter	Thermo Scientific	Eutech 1710	United States

3.4 Experimental Procedures and Formula

3.4.1 Sample Preparation and Extraction Process

The raw materials were dried in a food dehydrator under controlled conditions, using the *Clitoria ternatea* Wild Type and Mauve Type. Dried flowers were macerated by aqueous maceration protocol with solid to solvent ratio of 1:40 w/v for the extraction of pigments and polyphenols. Liquid extracts were separated from solids with Whatman No. 1 filter paper and stored in light-shielded refrigeration prior to being blended into defined operational treatment ratios. Extraction was carried out in a temperature-controlled water bath at 60°C for 30 min.

Table 3.4 Sample Extraction Ratio

Sample Code	Sample Ratio
W1	100% Wild Type
WM1	75% Wild Type : 25% Mauve Type
WM2	50% Wild Type : 50% Mauve Type
WM3	25% Wild Type : 75% Mauve Type
M2	100% Mauve Type

3.4.2 Moisture Content Analysis

The moisture content of the dried *Clitoria ternatea* flowers was determined using the official gravimetric oven-drying method (AOAC, 2005; Method 934.01). Porcelain crucibles were cleaned first, then dried in an oven, cooled in a desiccator and weighed to constant weight. Each crucible was then filled out with 2.00 g of the dried flower material and dried in a convective laboratory oven at 105 °C. When the difference

between two consecutive weighings was not greater than 0.005 g, which means that the final mass was constant, the drying was stopped. The crucibles were cooled in a desiccator containing silica gel and the final weights recorded so that the samples did not re-absorb moisture from the air.

The total moisture content was calculated as the percentage of mass lost during drying, using the following equation:

$$\text{Moisture Content (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \quad (1)$$

Where W1 is the initial weight of the sample before drying (g) and W2 is the final weight of the sample after drying (g). To ensure accuracy and analytical reliability, all measurements for this determination were performed in triplicate.

3.4.3 Color Profile

The extracts visual and spectral signatures were characterised using the UV-Vis spectrophotometer by recording the entire absorbance scans in the visible spectrum (400–700 nm at 1 nm intervals) according to the standard spectral scanning techniques for plant pigments (Skoog et al., 2017). Wild-dominant samples were diluted 1:10 v/v (1 mL of sample extract in 9 mL of distilled water) and Mauve-dominant samples were diluted 3:10 v/v (3 mL of sample extract in 7 mL of distilled water) in order to obtain clear peak resolution in the spectrophotometer. The instrument was zeroed with pure distilled water and the wavelength of maximum absorption (λ_{max}) and the peak absorbance values were measured in triplicates (n=3).

3.4.4 Total Anthocyanin Content (TAC)

The total anthocyanin content was determined by the Single-pH method, modified from the optical methods for monomeric anthocyanins (Lee et al., 2005; Marpaung, 2024). For sample preparation, 1 mL of the liquid extract was directly combined with 9 mL of a highly acidic potassium chloride buffer system (pH 1.0) to give a dilution

factor (DF) of 10 . This low pH environment compels the monomeric anthocyanins into their highly colored and stable flavylum cation form . Absorbance readings were measured using UV-Vis spectrophotometer at specific peak wavelength ($\lambda_{\max}$) for each of the five different formulation ratios. All sample extractions and measurements were performed in triplicate for reliability

The total anthocyanin content was calculated and expressed as milligrams of monomeric anthocyanin equivalents per liter (mg/L) using the following equation:

$$\text{TAC(mg/L)} = \frac{A \times \text{MW} \times \text{DF} \times 10^3}{\epsilon \times l} \quad (2)$$

Where:

A = optical absorbance value measured at the maximum wavelength ($\lambda=524$ nm)

MW = molecular weight of the dominant anthocyanin marker
(delphinidin-3-glucoside equivalent = 465.2 g/mol)

DF = operational volumetric dilution factor used during sample preparation

ϵ = molar absorptivity coefficient (delphinidin-3-glucoside = 29,000 L/mol · cm)

l = path length of the quartz spectrophotometer cuvette (1.0 cm)

10^3 = conversion factor from grams to milligrams

3.4.5 Total Phenolic Content (TPC)

The total phenolic content was determined colorimetrically, according to the Folin-Ciocalteu method (modified from procedures in the literature: Singleton & Rossi, 1965). In alkaline medium the polyphenols present in the extract reduce the phosphomolybdic-phosphotungstic acid complexes of the Folin-Ciocalteu reagent to a bright blue complex by an electron transfer process. This stable blue complex was measured by UV-Vis spectrophotometer at the detection wavelength of 765 nm . A standard calibration curve was prepared from known concentrations of pure gallic

acid to determine the concentration. All extractions and optical measurements were run in triplicate to guarantee data accuracy.

The quantitative values for TPC were calculated from the linear regression equation of the gallic acid calibration curve and expressed as milligrams of Gallic Acid Equivalents per liter of extract (mg GAE/L) using the following equation:

$$\text{TPC (mg GAE/L)} = C \times DF \quad (3)$$

Where:

C = The concentration of gallic acid equivalents (in mg/L or µg/ml) calculated from the calibration curve.

DF = The dilution factor of the sample (if no dilution was made, DF = 1).

3.4.6 Antioxidant Activity (DPPH Free-Radical Scavenging Assay)

The radical scavenging ability of the flower mixtures was evaluated by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free-radical assay according to the standard method of Brand-Williams et al. (1995). All sample extracts were tested at a uniform dilution factor of 70 (DF=70) for initial screening and comparative evaluation to allow a controlled and accurate functional comparison. Diluted sample aliquots were well mixed with a standard methanolic solution of deep purple DPPH radicals. It was incubated in dark for a certain period of time and the intensity of the color was measured at its maximum absorption at 517 nm using a UV-Vis spectrophotometer. All sample preparations and optical measurements were performed in triplicate for reliability.

The antioxidant activity was calculated and expressed as the Percentage of DPPH Radical Inhibition (%) using the following equation:

$$\text{Inhibition (\%)} = \left(\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100 \quad (4)$$

Where:

Abs_{blank} = absorbance value of the negative control (methanolic DPPH solution without extract)

Abs_{sample} = absorbance value of the operational sample mixture after the standard incubation period

3.4.7 Consumer Sensory Validation Framework and Protocol

A consumer acceptance test was chosen for this study in order to directly measure if the beverage formulation is acceptable to everyday consumers. This approach is crucial, because the beverage's main objective is to be commercially viable in the mid-market segment.

A sensory evaluation was conducted as a two factor test for colour preference and overall consumer acceptance. The panel consisted of 80 untrained consumer panellists. Testing occurred in a quiet, controlled room with neutral white lighting and a comfortable room temperature (24°C) to avoid environmental distractions or bias.

Each panellist was seated individually and provided with 20 mL samples of the various beverage formulations. The samples were served at the same time in identical, transparent, coded plastic cups and were cooled at 4°C. The two sensory attributes were rated by the panellists on a 9-point hedonic scale, with 1 being "Dislike Extremely," 5 being "Neither Like nor Dislike," and 9 being "Like Extremely." Panellists had to rinse their mouths with plain mineral water between each sample to cleanse their palate and make sure the scores were accurate and unbiased.

The most liked formulation (Sample 617/BD) was deeply characterised physico-chemically in a controlled laboratory environment after the sensory evaluation. This involved a range of analyses including Color Intensity wavelength scanning and total phenolic content measurements, with analytical precision ensured by filtering out solid jelly fractions prior to optical tests and performing analyses in

triplicate (n=3).

3.5 Data Analysis Techniques

Comparisons of statistical parametrics Single Factor Analysis of variance (ANOVA) was applied to the data for the 5 different ratios of flower extracts. Post hoc tests were performed to determine statistically significant differences among means at a confidence interval of $\alpha=0.05$ (Srinivasan and Montgomery, 1992).

Non-Parametric Sensory Statistics Sensory scores were obtained by using a 9-point hedonic scale were ordinal data and were analysed using non parametric statistics. The Friedman Test (df=4) was applied to identify the differences between the beverage variants globally at a significance level of $\alpha=0.05$ (Meilgaard et al., 2016). Preference grouping codes (a, b) were then obtained via pairwise comparisons using a localised Wilcoxon Signed-Rank Test.