Food Research 8 (Suppl. 7) : 59 - 66 (2024)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

Anthocyanin stability and antioxidant capacity of *Clitoria ternatea* incorporated jelly

[1,2,*]Zaidan, U.H., [1]Kamaruzaman, N.S., [2]Azhari, F. and [3]Gani, S.S.A.

[1]*Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[2]*Halal Products Research Institute, Universiti Putra Malaysia, Putra Infoport, 43400 UPM Serdang, Selangor, Malaysia*
[3]*Department of Agriculture Technology, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

Article history:
Received: 30 June 2024
Received in revised form: 12 August 2024
Accepted: 24 August 2024
Available Online: 9 December 2024

Keywords:
Clitoria ternatea,
Anthocyanin,
Antioxidant,
Temperature,
Sugar,
C. ternatea-incorporated jelly

DOI:
https://doi.org/10.26656/fr.2017.8(S7).8

Abstract

Antioxidants from natural sources from *Clitoria ternatea* (butterfly blue pea flower) have recently received great attention as an increase in oxidative stress disease is concerning. One of the most useful bioactive compounds from *C. ternatea* extract is anthocyanin, which is generally unstable due to various factors. Therefore, the extraction process needs to be optimized with a condition that favours the stability of anthocyanin. The effect of sugar as a preservative on the anthocyanin stability and antioxidant capacity of *C. ternatea* has been studied on anthocyanin-rich jelly formulated in two different conditions using dried butterfly blue pea flowers. The extract (E), jelly with sugar (JWS), and jelly without sugar (JWOS) were analyzed for their anthocyanin stability and antioxidant capacity for 11 weeks at two storage temperatures (25°C and 4°C). Total anthocyanin content was determined by the pH differential method, while antioxidant capacity was determined by a common 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. After two weeks of storage, the total anthocyanin content in the extract and jelly samples decreased over time, with no major difference between the samples in temperature and the presence of sugar. However, the extract alone possessed a higher and better stability of anthocyanin content (56.27 µg/mL to 7.19 µg/mL) after 14 days of storage at both temperatures (25°C and 4°C) compared to the processed food. It might be due to the processing effect on jelly samples during the analysis that might influence the final anthocyanin content (TAC). On the other hand, jelly incorporated with anthocyanin gives a significantly higher and more stable free radical scavenging activity than the extract, with no remarkable difference between jelly with sugar and without sugar at a different temperature. In addition, anthocyanin pigments from extract and jelly samples steadily degrade during storage at 25°C after 35 days of storage, which dramatically impacts colour quality and may also affect its nutritional properties. In conclusion, their food preparation (in extract or processed form) and storage temperature highly affected anthocyanin stability and its antioxidant capacity.

1. Introduction

The current lifestyle of commonly consuming processed foods, lack of exercise, and exposure to a wide range of toxins may lead to a condition defined as oxidative stress. It is marked by an imbalance in the formation and buildup of reactive oxygen species (ROS) in cells and tissues, with the biological system's inability to detoxify these reactive products that worsen the situation (Sharifi-Rad *et al*., 2020). From another point of view, modernization also brings a new chapter to technological and scientific innovation, which helps researchers study oxidative stress. As a result, we can now exploit antioxidant properties from many sources and synthetically produce antioxidant supplements to reduce the damage done by reactive molecules. However, not only did antioxidant supplements fail to help prevent diseases, but other issues arose regarding synthetic antioxidants. As summarized by Henkel *et al*. (2019), certain studies have described the negative consequences of antioxidant supplement consumption

*Corresponding author.
Email: *uswatun@upm.edu.my*

eISSN: 2550-2166
Published by Rynnye Lyan Resources

while demonstrating inconsistent positive outcomes.

Surprisingly, one of the unique plants known as *Clitoria ternatea* has been studied for its antioxidant capacity. *Clitoria ternatea* plant, commonly known as Asian pigeonwings or butterfly pea, is well-known for its blue colour. It has been widely used in cooking as a natural food colouring, particularly in Southeast Asia. Despite its distinctive blue colour, Swathi *et al.* (2021) mentioned that *C. ternatea* contains bioactive compounds such as phenols, flavonoids, alkaloids, glycosides, resins, steroids, saponins, and glycosides. Due to its rich bioactive chemical compounds, *Clitoria ternatea* has several benefits in the agricultural industry and medical applications despite serving as food colouring. The deep blue colour of this plant is believed to come from a pigment known as anthocyanin. Anthocyanin is not only responsible for the colour but also serves as one of the natural antioxidants and has been extracted for study from various sources, including *C. ternatea*. Thuy, Minh, Ben *et al.* (2021) reported that this plant has five anthocyanins; based on the intensity, cyanidin-3-glucoside was the most prevalent.

Thus, for the past few years, researchers have already aimed for the best extraction method and storage condition to get the highest yield of anthocyanin extract from *C. ternatea* and preserve its antioxidant capacity. One of the famous methods to maintain the stability of anthocyanin is sugar treatment. A study by Song *et al*. (2018) implied that sugars may be advantageous for preserving colour and anthocyanin stability, which depends on the sugar type, concentration, and structure. Furthermore, various kinds of sugar may have varying impacts on anthocyanin stability, and a higher quantity of sugars added may result in more stable anthocyanin pigment. Sugar's beneficial effect on anthocyanin stability could be attributed to a decrease in water activity, as sugar molecules are good at binding water molecules (Vukoja *et al*., 2019)

Anthocyanin extract from *C. ternatea* can be maximized by considering the main factors affecting its stability. This research also hypothesized that sugar treatment on anthocyanin extract may preserve the pigment's stability during storage, thus contributing to its antioxidant capacity. Therefore, the objectives of this study are to investigate the effect of processing methods on the stability of anthocyanin and antioxidant capacity, to evaluate the effect of storage conditions on the stability of anthocyanin and antioxidant capacity, and to evaluate the relationship between storage time and anthocyanin stability and its antioxidant capacity by using Pearson correlation analysis.

2. Materials and methods

2.1 Sample preparation

Clitoria ternatea dried flower purchased was ground into powdered form by using a drying blender. The powdered flower was kept in an opaque and sealed container at refrigerator temperature (4°C) in dark condition.

2.2 Extraction of Clitoria ternatea plant

This study chose the non-conventional method, ultrasonic-assisted extraction (UAE), according to Thuy, Ben, Minh *et al*. (2021). Approximately 10 g of ground dried pea flowers were mixed with 250 mL of distilled water in a 250 mL conical flask to start the extraction process. UAE was conducted in an ultrasonic bath for 30 minutes at 70°C. The extract was then filtered into a beaker using Whatman filter paper (Whatman no. 4). This step was repeated until the extract reached approximately 1 L volume. Then, the extracted stock was sent to freeze-dry and stored at the same condition as sample preparation for further processing. Extract samples kept at 25°C and 4°C for analysis were denoted as E25 and E4.

2.3 Preparation of Clitoria ternatea jelly

Jelly processing was conducted according to an established procedure with a slight adjustment. Conventional *C. ternatea* jelly was prepared: 0.5 g of powdered sample flower mixed with 500 mL of distilled water. Then, 20% (w/v) of sucrose solution, 5 g agar-agar powder, and 25 g gelatin were added. The mixture was heated in a gas stove until ebullience boiling for 15 mins and cooled at room temperature. The jelly was hot packed in glass jars and stored for 11 weeks at 4°C and 25°C. Another set of samples was prepared with the same procedure but without sucrose solution added to investigate the effect of preservatives on anthocyanin stability. All samples were prepared in duplicate and analyzed every 7 days shortly after storage. Jelly samples with sugar kept at 25°C and 4°C were denoted as JWS25 and JWS4; meanwhile, jelly without sugar was denoted as JWOS25 and JWOS4.

2.4 Extraction of anthocyanin from Clitoria ternatea jelly

A total of 5 g jelly was extracted, weighed, and mixed with 25 mL of 80% aqueous ethanol with 1% concentrated hydrochloric acid. The extraction was done using a rotary shaker at 150 rpm and 55°C for 30 mins. Next, the extract was filtered using Whatman filter papers (102 qualitative; diameter 125 mm, A0336), and the filtrate was used to measure total anthocyanin content (TAC) and DPPH free radical scavenging activities.

https://doi.org/10.26656/fr.2017.8(S7).8

2.5 Total anthocyanin content analysis

Total anthocyanin content was measured using the pH differential method described by Teng *et al.* (2020) and Lee *et al.* (2005). The sample was adjusted to reach pH 1 and pH 4.5 by the addition of a buffer prepared beforehand. The absorbance of diluted samples was measured in duplicate at the highest peak in the visible region (absorbance at λmax), which is 520 nm, with ethanol as the blank. The standard curve of anthocyanin concentration versus absorbance was constructed with R2 = 0.9807, showing good linearity for the standard. This study chooses cyanidin-3-glucoside as the standard because it is the most common anthocyanin found in nature, and most studies related to *C. ternatea* also used this standard. The range of concentration used for the standard was 0, 5, 10, 20, 60, 80 and 100 µg/mL.

2.6 Antioxidant activity analysis

Analysis of antioxidant capacity was done by DPPH assay with the steps used referring to the Sofyan *et al.* (2022) study with some adjustments. First, 12 mg of DPPH powder (Molar mass = 394.33) was diluted into 300 mL ethanol (96% V/V) to produce 100 µM DPPH stock solution. The next step was adapted from Vukoja *et al.* (2019), with slight modification where 0.2 mL of sample was mixed with 2.8 mL of DPPH stock solution (100 µM) to the final volume of 3 mL. After 30 min of incubation in the dark, the absorbance was measured at 517 nm in duplicate. Lastly, the percentage of inhibition and antioxidant activity was evaluated using the equation below:

$$\% \text{ RSA/Inhibition} = ([ADPPH - AS] / ADPPH) \times 100$$

Where ADPPH is the absorbance of control (DPPH + ethanol) and AS is the absorbance of sample (DPPH + sample).

2.7 Colour change

The naked eye observed extract and jelly samples at both temperatures and pictures were taken for data collection throughout 77 days of storage at one-week intervals.

2.8 Statistical analysis

Statistical analysis was conducted using the IBM SPSS Statistic Version 26.0. The correlation between storage time, total anthocyanin content, and total antioxidant activity was analyzed using Pearson's correlation.

3. Results and discussion

3.1 Total anthocyanin analysis

The total anthocyanin content was analyzed using the pH differential method. The fundamental idea behind determining the amount of anthocyanin is that anthocyanin changes structurally depending on the pH, showing a rich reddish colour at pH 1.0. It forms a colourless hemiketal form at pH 4.5 (Teng *et al*., 2020). For better understanding, all components in the sample, including anthocyanins, absorb 520 nm light, thus responsible for the absorbance values observed at a pH of 1.0. Meanwhile, absorbance values at pH 4.5 are only attributable to the other compounds and not to anthocyanin due to its structural changes.

Figure 1 shows the declining graph profile for 77 days of storage with processed food having negative total anthocyanin content (TAC) values for both samples with sugar and without sugar at both temperatures (25°C and 4°C). Overall, extract alone at both temperatures has a higher TAC content profile, followed by jelly with sugar at both temperatures and jelly without sugar has the lowest TAC despite the temperature difference. The correlation coefficient value of this study is significant to indicate that storage time did have an impact on the concentration of total anthocyanin content. As described in Table 1, both extract samples at 25°C and 4°C have high negative correlation values (near -1), showing a strong correlation between the storage time and total anthocyanin content. In other words, as the storage time increases, total anthocyanin drastically decreases in the extracted sample. Meanwhile, all anthocyanin-incorporated food (jelly) shows a moderate negative correlation regardless of the presence of sugar and storage temperature. Again, this study can conclude that the moderate decline of total anthocyanin content in this processed food correlates with increased storage time. Shinwari and Rao (2018) state that degradation of bioactive compounds such as anthocyanin may continue during storage depending on storage conditions, with storage temperature and duration having a major effect. This study assumes that the processing technique

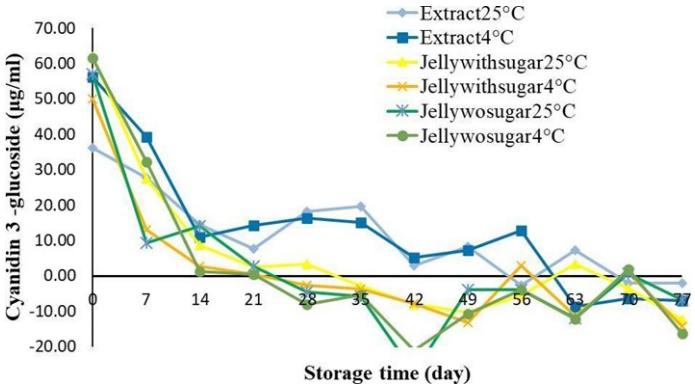

Figure 1. Concentration of anthocyanin (µg/mL) in all samples for 77 days of analysis.

https://doi.org/10.26656/fr.2017.8(S7).8

enhances the anthocyanin stability rather than the extract alone, as the extract samples experience greater loss of the anthocyanin content than jelly samples at the same storage period.

Despite the graph profile shown, correlation analysis does meet the hypothesis and expectation of this study even theoretically; jelly with sugar and without sugar at both temperatures should have a higher TAC content profile in Table 1 compared to the extracted sample. It is assumed that this might be due to the jelly extraction process prior to the assay step. An additional step probably influences the structure of anthocyanin pigment as anthocyanin is sensitive to pH changes. The negative value of total anthocyanin content might also result from this case. Fu *et al*. (2021) support the finding that *C. ternatea* extract does decline with the increase in temperature, and they concluded that the extract can be stored stably for months at 4°C in a neutral pH range (4–8).

Jelly extraction prior to the pH differential assay used 80% ethanol and 1% HCl at 55°C for 50 mins, resulting in changes in the jelly extract colour from blue to pink. When anthocyanins interact with acids and bases, they go through molecular changes. The flavylium ion, which produces a red colour, is typically the dominant form in an acidic media. Meanwhile, anhydrobase anion is formed by anthocyanin at a slightly higher pH (2–4), giving it a bluish colour. It also explains why the sample changed colour when buffer pH 1 and buffer pH 4.5 were added. Enaru *et al*. (2021) mentioned that prolonged thermal treatment also caused the opening of the anthocyanin structure's pyrylium ring, resulting in chalcone production, which was accountable for colour changes in the jelly extract. However, lowering the anthocyanin solution's pH can counteract the downside of heat treatment.

Moreover, adding the buffer to the pink colour jelly sample will result in a paler pink colour. Concurrently, some samples stored at 25°C lose colour as the storage time increases. Eventually, adding the buffer makes the sample's colour fade more, even with a similar dilution ratio. As a result, diluted samples give lower absorbance readings. Beer's Law supported the result, which explains that the amount of light absorbed will depend on the number of molecules interacting with the light. In an exceedingly diluted solution, it may be difficult to see any colour; therefore, the absorption will be quite low (Wypych, 2020). It explains why extract samples have a higher TAC content profile compared to jelly samples. Appropriate jelly extraction method and suitable dilution ratio should be determined beforehand to maintain the colour intensity during absorbance reading.

Proper dilution factor can be determined by testing the sample with pH 1.0 buffer until the absorbance at 520 nm is within the spectrophotometer's linear range. The absorbance for most spectrophotometers should be between 0.2 and 1.4 AU. Some studies measure the absorbance at 700 nm as well, but according to Lee *et al*. (2005), it is to correct for haze. If the diluted test portion is excessively turbid, the sample must be clarified by centrifuging or filtering before the absorbance reading.

In addition, this study is interested in discussing the drawbacks of using the pH differential method to determine the total anthocyanin content from *C. ternatea*. Despite being an easy and convenient method, it can only estimate total anthocyanins based on a chosen anthocyanin equivalent, which commonly, cyanidin-3-glucoside is used. Moreover, the pH differential method is inappropriate for some plants like *C. ternatea*. Since *C. ternatea* has an acyl group located at the ring B, Marpaung and Tjahjadi (2019) reported that the colour intensity of *C. ternatea* extract at pH 4 was higher than at pH 1. The higher intensity was possible because purple and blue colours absorb light more intensely than red, and at pH 4, *C. ternatea* extract exhibited blue to blue-purple colours. Hence, the pH differential concept does not fit well with the anthocyanin absorbance profiles from *C. ternatea.*

One reliable method commonly used for anthocyanin quantification is high-performance liquid chromatography (HPLC). The HPLC method is based on the chromatogram's anthocyanin peak area, which is indirectly proportional to the anthocyanin concentration. It indicates that the HPLC approach for qualitative analysis can also be used for anthocyanin quantification. Even though anthocyanin profiles vary from each type of fruit or plant, Teng *et al*. (2020) said that HPLC can quantitatively and reliably evaluate both total and

Table 1. Pearson correlation analysis between total anthocyanin content and storage time.

Sample	Correlation	Interpretation	Explanation
E25	-0.854	Strong negative	As the day increases, TAC decreases strongly
E4	-0.861	Strong negative	As the day increases, TAC decreases strongly
JWS25	-0.742	Moderate negative	As the day increases, TAC decreases moderately
JWS4	-0.689	Moderate negative	As the day increases, TAC decreases moderately
JWOS25	-0.631	Moderate negative	As the day increases, TAC decreases moderately
JWOS4	-0.692	Moderate negative	As the day increases, TAC decreases moderately

https://doi.org/10.26656/fr.2017.8(S7).8

individual anthocyanins in the solution. This study would like to suggest HPLC as one of the tools to measure the anthocyanin content in plants like *C. ternatea* until a validated anthocyanin standard is available.

3.2 Total antioxidant capacity

In the theory of antioxidant assay, visually noticeable colour changes will appear when free radicals react with an antioxidant. This study chooses the DPPH assay on the base that reducing the violet DPPH radical by the antioxidant through a hydrogen atom transfer mechanism will change colour to stable light yellow DPPH molecules (Sirivibulkovit *et al*., 2018). Then, a UV-Vs spectrophotometer is used to quantify the remaining violet DPPH free radical between 515 and 520 nm to calculate the antioxidant activity. The overall result for six samples is shown in Figure 2. There is a large gap in the percentage of free radical inhibition between extract samples and processed food (jelly) samples. As shown in the graph, both extracts at 25°C and 4°C inhibit at a maximum of 25%. This study also found that free radical scavenging activity is much higher in anthocyanin-incorporated food, with almost 80% inhibition as the storage time increases. Nevertheless, free radical scavenging activity in this sample type eventually decreases after some time, specifically after 56 days of storage. There is not much difference between the presence of sugar and storage conditions in maintaining the antioxidant activity of the *C. ternatea* jelly sample.

Also, Pearson's correlation analysis explains that only jelly kept at 4°C (jelly with sugar and jelly without sugar) has a significant correlation coefficient value (Table 2). Thus, only the correlation for this type of sample is reliable, and it can be concluded that the percentage of free radical inhibition gradually decreased as the storage time increased. In contrast, samples other than these two have insignificant correlation coefficient values, which leads to objections to the correlation analysis. In these samples, decreasing the percentage of DPPH inhibition might just happen from chance, and storage time can be assumed not to influence it. As the p-value is larger than 0.05, this study failed to reject the null hypothesis, implying that a correlation does not exist between total antioxidant activity and storage time in extract samples at both temperatures, jelly with sugar and jelly with sugar at 25°C.

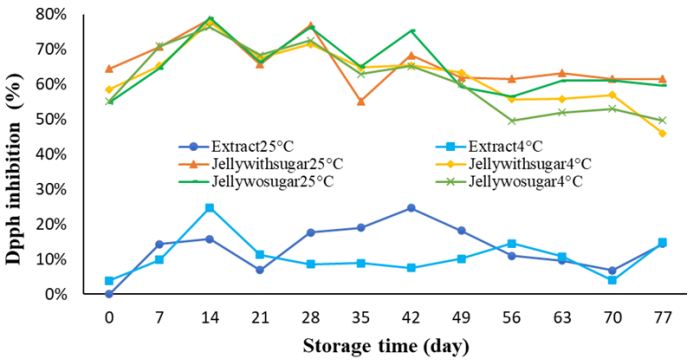

Figure 2. Free radical scavenging activity in all samples against storage time.

Besides that, this study highlights that the antioxidant activity of anthocyanin from *C. ternatea* followed the expected DPPH profile trend for all six samples. However, it does not parallel the total anthocyanin analysis result previously. The differences between the total anthocyanin and antioxidant activity obtained in this study are due to the TAC analysis assay steps involving additional solvent used, different operational conditions (temperature and time), and other reasons discussed in detail in the early part of this discussion. Contrastingly, the DPPH assay is a direct method where the DPPH stock solution was mixed with the sample, incubated for 30 mins, and an absorbance reading was taken. No additional solvent, neither time nor temperature, is being adjusted. Hence, the free radical scavenging profile for all six samples is acceptable in this study as *C. ternatea* has many other bioactive compounds besides anthocyanin that can serve as antioxidants (Ramli *et al*., 2021).

Overall, the processing method to incorporate anthocyanin extract into jelly enhanced and maintained the antioxidant activity higher and longer than the extract alone. Adding sugar and jelly ingredients such as pectin played a role in preserving the antioxidant effect. As described by Shinwari and Rao (2018), both substances have been observed to stabilize the anthocyanins by absorbing the flavylium cation. It is known that sugars can reduce water activity and shield the flavylium cation

Table 1. Pearson correlation analysis between total anthocyanin content and storage time.

Sample	Correlation	Interpretation	Explanation
E25	0.124	No association	Day does not correlate with percentage inhibition
E4	-0.02	No association	Day does not correlate with percentage inhibition
JWS25	-0.495	Weakly negative	As the day increases, percentage inhibition decreases eventually
JWS4	-0.667*	Moderate negative	As the day increases, percentage inhibition decreases moderately
JWOS25	-0.315	Weakly negative	As the day increases, percentage inhibition decreases eventually
JWOS4	-0.695*	Moderate negative	As the day increases, percentage inhibition decreases moderately

*Correlation is significant at the 0.05 level (2-tailed).

https://doi.org/10.26656/fr.2017.8(S7).8

from nucleophilic attacks. A study on grape stem extracts by Jiménez-Moreno *et al*. (2019) concluded that other chemicals besides polyphenols had antioxidant properties besides those that were extractable from the samples. Thus, this study also assumed that the Maillard reaction's byproducts in jelly samples could be a factor that contributes to its antioxidant activity.

3.3 Colour change

A common phenomenon was observed regarding the colour variation of *C. ternatea* extract and its jelly formulated during the studied period (11 weeks). Figure 3 shows the effect of preservative and storage temperature on the stability of *C. ternatea* pigments.

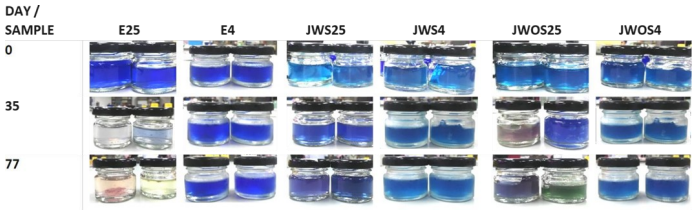

Figure 3. Colour observation of extract and jelly samples at 0, 35 and 77 days.
Note: E25, JWS25 and JWOS25 are notated as extract, jelly with sugar and jelly without sugar kept at 25°C, respectively. E4, JWS4 and JWOS4 are extracts, jelly with sugar and jelly without sugar kept 4°C, respectively.

Notably, the blue colour of *C. ternatea* extract kept at 25°C (E25) initially changed from intense blue to light blue and finally to pale yellow and almost colourless after 77 days of storage. Moreover, there is a brown precipitate formation, mostly found as the final product of the thermal effect of anthocyanin extracts of fruits and flowers. Meanwhile, the extract samples kept at 4°C (E4) experienced little to almost no degradation of blue pigment throughout 77 days of storage, indicating that the anthocyanin was stable under the provided condition. Shinwari and Rao (2018) also observed a similar finding that most berry fruit jelly or jam stored at room temperature have greater loss of their bioactive compound.

Moreover, all jelly samples were kept at 4°C regardless of preservatives (JWS4 and JWOS4), successfully maintaining the textures' viscosity as jelly food for 77 days of analysis. Thus, this finding was contradictory with jelly samples kept at 25°C (JWS25 and JWOS25), which became watery due to the spoilage effect after several weeks of storage. Also, jelly with sugar samples at 25°C (JWS25) has slight colour differences, while jelly without sugar samples (JWOS25) showed drastic undesirable colour changes with mold growth. Due to the complexity of processed food such as jelly, many variables can affect how bioactive molecules alter throughout the processing method. In this study, the presence of sugar has a significant role in the stability of anthocyanin.

The presence of sugar did enhance the stability of anthocyanin and antimicrobial properties, as demonstrated by slower changes in colour fading and the visual appearance of jelly with sugar samples during storage at 25°C. According to Chatterjee and Abraham (2018), foods with a high sugar content are not commonly spoilt because of additional sucrose or glucose. Fructose and sucrose are two sugars that work well at preventing the growth of bacteria that cause food spoilage. Since samples are kept at optimum temperature for microbial growth, known as the 'danger zone,' contamination on the sample might degrade the anthocyanin pigment and influence the analysis results. It is reported by Enaru *et al*. (2021) that enzymes that can break down anthocyanins, such as glycosidases, peroxidases (phenol oxidases), and phenolases, may be produced by microbial contamination. It is important to note that peroxidases and phenolases indirectly affect the stability of anthocyanins, while glycosidases directly damage them.

3.4 Novel food grade preparation and storage condition improvement

Having a controlled environment during food processing is crucial enough to ensure the shelf life of food products, especially when it involves an unstable bioactive compound. Proper handling and treatment can sustain the food quality longer, and it is also necessary to consider the physicochemical characteristics of the product in which the natural pigments will be incorporated. This study noted that stabilizing anthocyanin from *C. ternatea* is the main challenge before it can serve as a food colourant. Hence, a few parts of *C. ternatea* jelly preparation will be addressed in this section.

Most food production procedures rely on minimal contamination from the production environment to produce safe and high-quality products. Disinfection, rinsing, and drying processes attempt to reduce the number of microorganisms to a suitable level compatible with safe food manufacturing (Møretrø and Langsrud, 2017). Because jelly is grouped as perishable food, it is recommended to maintain the surface hygiene of the glass jar and other equipment used beforehand while keeping the best condition for pigment's stability. Other than avoiding anthocyanin degradation by an enzyme released from the microbes, it also ensures that the jelly can look pleasurable for longer. Nevertheless, storage temperature did have the biggest influence on microbial growth and anthocyanin stability in this study.

https://doi.org/10.26656/fr.2017.8(S7).8

Next, lowering the pH of *C. ternatea* jelly can also help maintain the anthocyanin stability for a long time. Two studies with anthocyanin from blueberry (Liu *et al*., 2018) and red rose (Wahyuningsih *et al*., 2017) concluded that lower pH (acidic condition) is beneficial towards anthocyanin stability. At this pH (pH<6), anthocyanin will exhibit a red-pink colour as the cyanidin molecule will be protonated and form a positive ion or flavylium cation, which exhibits a red-to-pinkish colour. Theoretically, these colour changes do not affect the jelly's sensory evaluation much. From blue to pink, it is still categorized as acceptable and suitable for a functional food product. However, since the *C. ternatea* incorporated jelly in this study was prepared as homemade or self-made jelly, lowering pH would bring a sourer taste and affect consumer preferences.

Lastly, the storage condition of *C. ternatea* jelly needs to be evaluated again to understand and presume how long anthocyanin from *C. ternatea* extract can be stable in a functional food model. Other than temperature and presence of preservatives as conducted in this study, factors such as light should also be focused on. As summarized by Enaru *et al*. (2021), the presence of light can accelerate bioactive decomposition. Surprisingly, anthocyanin degradation is highest when pigments are exposed to fluorescent light. This study exposes anthocyanins to light throughout the process, from extraction stages to jelly processing and storage periods. Moreover, the result of this study already shows that samples kept at room temperature have the most pigment degradation after 77 days of analysis. Hence, any functional food incorporated with *C. ternatea* extract should be stored in dark conditions to sustain the pigment longer.

4. Conclusion

Concisely, *C. ternatea* extracts did have a notable anthocyanin content and antioxidant capacity that was proven by much research. The variety of experimental conditions in extraction techniques, analyzing parameters, and quantification makes it difficult to choose the best framework for attaining the most efficient anthocyanin recovery. Therefore, the standardization of extraction and analytical techniques may be essential to enable a precise evaluation of these experimental outcomes. After 11 weeks of analysis, the total anthocyanin content in both extract and jelly samples decreased over time, with the anthocyanin extract possessing a more stable anthocyanin content than the jelly form. Meanwhile, concerning antioxidant activity, jelly-incorporated anthocyanin demonstrated a higher and more stable free radical scavenging activity than the extract samples. There was no remarkable difference between jelly with and without sugar at a different temperature. In addition to colour observation, all samples during storage at 4℃ may preserve the colour and texture. Concurrently, anthocyanin pigments from extract and jelly samples steadily degrade during storage at 25℃, dramatically impacting colour quality and nutritional properties.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors gratefully acknowledge the financial assistance grant funded by Geran Inisiatif Putra Siswazah, Universiti Putra Malaysia (Grant No. 9701100).

References

Chatterjee, A. and Abraham, J. (2018). Microbial contamination, prevention, and early detection in food industry. In Holban, A.M. and Grumezescu, A.M. (Eds.) Microbial Contamination and Food Degradation, p. 21-47. USA: Academic Press. https://doi.org/10.1016/B978-0-12-811515-2.00002-0

Enaru, B., Drețcanu, G., Pop, T.D., Stănilă, A. and Diaconeasa, Z. (2021). Anthocyanins: Factors affecting their stability and degradation. *Antioxidants*, 10(12), 1967. https://doi.org/10.3390/antiox10121967

Fu, X., Wu, Q., Wang, J., Chen, Y., Zhu, G. and Zhu, Z. (2021). Spectral characteristic, storage stability and antioxidant properties of anthocyanin extracts from flowers of butterfly pea (*Clitoria ternatea* L.). *Molecules*, 26(22), 7000. https://doi.org/10.3390/molecules26227000

Henkel, R., Sandhu, I.S. and Agarwal, A. (2019). The excessive use of antioxidant therapy: a possible cause of male infertility? *Andrologia*, 51(1), e13162. https://doi.org/10.1111/and.13162

Jiménez-Moreno, N., Volpe, F., Moler, J.A., Esparza, I. and Ancín-Azpilicueta, C. (2019). Impact of extraction conditions on the phenolic composition and antioxidant capacity of grape stem extracts. *Antioxidants*, 8(12), 597. https://doi.org/10.3390/antiox8120597

Lee, J., Durst, R.W., Wrolstad, R.E. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colourants, and wines by the pH differential method: Collaborative study. *Journal of AOAC International*,

88(5), 1269-1278. https://doi.org/10.1093/jaoac/88.5.1269

Liu, Y., Liu, Y., Tao, C., Liu, M., Pan, Y. and Lv, Z. (2018). Effect of temperature and pH on stability of anthocyanin obtained from blueberry. *Journal of Food Measurement and Characterization*, 12(3), 1744-1753. https://doi.org/10.1007/s11694-018-9789-1

Marpaung, M. and Tjahjadi, K.H. (2019). The analysis of monomeric anthocyanin by pH differential method is not appropriate for certain anthocyanins, presented at the 16th ASEAN Food Conference. Bali, Indonesia. https://doi.org/10.5220/0009985400260030

Moretro, T. and Langsrud, S. (2017). Residential bacteria on surfaces in the food industry and their implications for food safety and quality. *Comprehensive reviews in Food Science and Food Safety*, 16(5), 1022-1041. https://doi.org/10.1111/1541-4337.12283

Ramli, M.E., Salleh, R.M., Tajarudin, H.A. and Zulkurnain, M. (2021). Influence of amylose content on phenolics fortification of different rice varieties with butterfly pea (*Clitoria ternatea*) flower extract through parboiling. *Food Science and Technology*, 147, 111493. https://doi.org/10.1016/j.lwt.2021.111493

Sharifi-Rad, M., Anil Kumar, N.V., Zucca, P., Varoni, E.M., Dini, L., Panzarini, E. and Sharifi-Rad, J. (2020). Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. *Frontiers in Physiology*, 11, 694. https://doi.org/10.3389/fphys.2020.00694

Shinwari, K.J. and Rao, P.S. (2018). Stability of bioactive compounds in fruit jam and jelly during processing and storage: A review. *Trends in Food Science and Technology*, 75, 181-193. https://doi.org/10.1016/j.tifs.2018.02.002

Sirivibulkovit, K., Nouanthavong, S. and Sameenoi, Y. (2018). Paper-based DPPH assay for antioxidant activity analysis. *Analytical Sciences*, 34(7), 795-800. https://doi.org/10.2116/analsci.18P014

Sofyan, J., Permana, T. and Marpaung, A.M. (2022). The study of several applicable treatments for serving butterfly pea flower drinks. In Proceedings of the 6th International Conference of Food, Agriculture, and Natural Resource (IC-FANRES 2021), p. 50-56. USA: Atlantis Press International B.V. https://doi.org/10.2991/absr.k.220101.008

Song, H.N., Ji, S.A., Park, H.R., Kim, H.H. and Hogstrand, C. (2018). Impact of various factors on colour stability of fresh blueberry juice during storage. *Preventive Nutrition and Food Science*, 23 (1), 46. https://doi.org/10.3746/pnf.2018.23.1.46

Swathi, K. P., Jayaram, S., Sugumar, D. and Rymbai, E. (2021). Evaluation of anti- inflammatory and anti-arthritic property of ethanolic extract of Clitoria ternatea. *Chinese Herbal Medicines*, 13(2), 243-249. https://doi.org/10.1016/j.chmed.2020.11.004

Teng, Z., Jiang, X., He, F. and Bai, W. (2020). Qualitative and quantitative methods to evaluate anthocyanins. *eFood*, 1(5), 339-346. https://doi.org/10.2991/efood.k.200909.001

Thuy, N.M., Ben, T.C., Minh, V.Q. and Van Tai, N. (2021). Effect of extraction techniques on anthocyanin from butterfly pea flowers *(Clitoria ternatea* L.) cultivated in Vietnam. *Journal of Applied Biology and Biotechnology*, 9(6), 1-8. https://doi.org/10.7324/JABB.2021.96022

Thuy, N.M., Minh, V.Q., Ben, T.C., Thi Nguyen, M.T., Ha, H.T.N. and Tai, N.V. (2021). Identification of anthocyanin compounds in butterfly pea flowers *(Clitoria ternatea* L.) by ultra performance liquid chromatography/ultraviolet coupled to mass spectrometry. *Molecules*, 26(15), 4539. https://doi.org/10.3390/molecules26154539

Vukoja, J., Pichler, A. and Kopjar, M. (2019). Stability of anthocyanins, phenolics and colour of tart cherry jams. *Foods*, 8(7), 255. https://doi.org/10.3390/foods8070255

Wahyuningsih, S., Wulandari, L., Wartono, M.W., Munawaroh, H. and Ramelan, A.H. (2017). The effect of pH and colour stability of anthocyanin on food colourant. *IOP conference series: Materials Science and Engineering*, 193(1), 012047. https://doi.org/10.1088/1757-899X/193/1/012047

Weerasinghe, T., Perera, D., De Silva, N., Poogoda, D. and Swarnathilaka, H. (2022). Butterfly pea: An emerging plant with applications in food and medicine. *The Pharma Innovation Journal*, 11(6), 625-637.

Wypych, G. (2020). Handbook of UV degradation and stabilization. 3rd ed. Toronto, Canada: Elsevier.

