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Study Literature: Purple Cabbage (Brassica oleracea L) as a New Innovation Test Indicator for Biochemical Carbohydrate Fermentation

Winda Amalia[1], Inayah[1], Dhafa Satrio Rizki Wicaksana[1], and Oktavina Nikmatuz Zahra[1]

[1]Natural Science Education, Faculty of Mathematics and Natural Science, University State Semarang, Kampus Sekaran Gunungpati Kota Semarang 50229 Jawa Tengah, Indonesia.

*Correspondinge-mail: oktavinanz@students.unnes.ac.id

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Abstract: **Study Literature: Purple Cabbage (Brassica oleracea L) as a New Innovation Test Indicator for Biochemical Carbohydrate Fermentation.** Anthocyanins in purple cabbage can be dissolved in 96% alcohol. Under certain conditions, these anthocyanin compounds can experience color changes in acidic, alkaline, and neutral conditions. Utilization of purple cabbage carbohydrate fermentation test indicators, in the form of extracts. Which can be marked by a change in color, especially in the mannitol and lactose test media. The process of changing this color is from which initially there is no color at all to red. So with this in mind, some researchers concluded that purple cabbage extract could be used as an alternative color indicator for testing carbohydrate fermentation, especially mannitol and lactose.

Keywords: *purple cabbage, fermentation, indicators*

Abstrak: Kajian Literatur : Kubis Ungu (Brassica oleracea L) sebagai Inovasi Baru Indikator Uji Fermentasi Karbohidrat Biokimia. *Antosianin ditemukan dalam kubis ungu dan dapat dilarutkan dalam 96% alkohol. Dalam kondisi tertentu yaitu dalam keadaan asam, basa, dan netral senyawa Antosianin ini dapat mengalami perubahan warna. Pemanfaatan pada indikator uji fermentasi karbohidrat kubis ungu dimanfaatkan dalam bentuk ekstrak. Yang mana, dapat ditandai dengan adanya perubahan warna khususnya pada media uji manitol dan laktosa. Proses berubahnya warna ini adalah dari yang awalnya sama sekali tidak terdapat warna menjadi warna merah. Sehingga dengan adanya hal tersebut beberapa Peneliti menyimpulkan bahwa ekstrak kubis ungu dapat dijadikan sebagai alternatif lain indikator warna pengujian fermentasi karbohidrat khususnya manitol dan laktosa.*

Kata kunci*:* *kubis ungu, fermentasi, indikator*

▪ INTRODUCTION

This pigment is called anthocyanin, and it is found in many fruits and plants throughout Indonesia that give them their color. There are some situations in which the pH of the solution can have an impact on the color of anthocyanins. This pigment is considered to have the potential as a natural indicator because of its ability to change color (Riniati, 2019). Plants such as hibiscus (Hibiscus rosa sinensis L), Leunca (Solanum nigrum Linn), and Rhoediscolor are some examples of plant implementations which are generally used as natural indicators. Natural ingredients such as purple cabbage (Brassica oleracea L) contain anthocyanins in addition to these natural ingredients (Nurjanah, 2020).

Anthocyanins give cabbage its purple color, also known as purple cabbage. Anthocyanins are very well extracted in ethanol, giving a blue-purple color at 96%. In the neutral, acidic, and basic pH ranges, anthocyanins can change color (Nuroniyah, 2022). The fermentation of carbohydrates in a biochemical test medium can be used as an indicator of reactions by observing color changes at various pH levels.

The biochemical test medium is a mixture of food (nutrients) that is needed by microorganisms in order to survive through metabolism and to grow. An instrument used to observe the physiological and biochemical reactions of microorganisms, usually in liquid, semisolid, or solid form, and that may contain natural or synthetic ingredients (Jawetz and Melnick, 2013).

A large number of carbohydrates (mannitol or lactose), peptones, and indicators are included in the biochemical test medium for carbohydrate fermentation. As one of the chemical indicators used, bromcresol purple (BCP) is one of the most common. Upon changing the pH path on the media, the BCP indicator will change color according to the pH path, i.e. purple for the base and neutral pH path, and yellow for the acid pH path. Carbohydrates and peptone water are carbon sources. Meanwhile, BCP is an indicator of synthesis. If bacteria are able to ferment carbohydrates, they will form acids. The disadvantages of synthetic indicators such as BCP are their high cost and difficult availability. Alternative indicators Natural ingredients made from purple cabbage are relatively easy to obtain, less expensive, and change color depending on the pH.

Gustriani's previous research on the pH route of purple cabbage extract in ethanol extract solvent revealed that 95 percent ethanol concentration was the most effective ethanol solvent concentration. This is with an absorbance value of 0.3222 and a wavelength of 544 nm or more. The pH of purple cabbage ranges from 6.5-7.50 (blue-purple), 10.50-12.00 (bluish green), and 12.00-13.00 (green-yellow bluish).

Based on this background, an article was written regarding purple cabbage solution which can be used as an acid indicator in biochemical test media for mannitol and lactose fermentation. This solution can be used as a substitute for synthetic bromocresol purple indicators which are usually applied in mannitol and lactose carbohydrate fermentation media.

▪ METHOD

The method used in this paper is literature review. This paper discusses purple cabbage which is used as a test indicator in biochemical carbohydrate fermentation which is a new innovation. To analyze this, we searched article databases (google scholar and SPRINGER). The steps taken to identify purple cabbage as an indicator in the biochemical carbohydrate fermentation test are: (1) Analyze the content of purple

cabbage (2) Analyze the experiments conducted on purple cabbage extract (3) Finally, the conclusions are presented in this review.

▪ RESULT AND DISCUSSION

Purple cabbage extract has a distinctive color from anthocyanins which are dark purple, where the color is a natural pigment and has a significant color change at each level of change in pH from acidic to neutral and alkaline (Puspita et al., 2021).

Suhartati (2021) conducted an experiment with 2 treatments, namely in the first treatment, the purple cabbage extract indicator was added to the media composition before being inoculated with bacteria. For the second treatment, purple cabbage extract indicators were added to the media that had been inoculated with bacteria. Suhartati used the BCP indicator of 0.04% as a test control.

Inoculum according to Djamaan (2016) is a substance containing microorganisms/other materials that are included in the inoculation process. Inoculum (starter) used in the fermentation industry must meet several criteria, namely: the microbial culture must be in an active state, must be available in sufficient quantities to achieve optimal proportions of inoculum and fermentation media, must be free of contaminants, and the ability to form products must be stable.

BCP (Brom Cresol Purple) was still used as a control to compare alternative indicators of purple cabbage with indicators usually used for fermentation. Maghfira (2020) also used the BCP indicator in his research inoculating bacteria in the carbohydrate fermentation test. A positive reaction from carbohydrate fermentation is indicated by the formation of gas and acid bubbles which are indicated by purple and yellow color changes. The steps taken by Suhartati (2021) in treatments 1 and 2 are as follows:

1. Prepare 3 test tubes (2 tubes for mannitol media, 1 tube for lactose medium).
2. Put mannitol and lactose media in each test tube then add 4.5 mL of purple cabbage solution each.
3. Carry out the autoclave process.
4. Cools the media.
5. Embedding S.aureus and E.coli bacteria on mannitol media. Implant the bacteria Lactobacillus casei.
6. Perform incubation processing of prepared mannitol and lactose media. Bacteria were grown at 37°C for 24 hours.
7. Test the color indicators in the mannitol and lactose fermentation test by adding a few drops of purple cabbage solution to the mannitol and lactose media.

Tabel 1. Hasil Uji Biokimia Perlaukan 1 Indikator terkandung dalam media

Uji Biokimia	Kode	Jenis indikator	Jenis bakteri	Pertumbuhan bakteri	Perubahan indikator
Kontrol	B1.1	BCP 0,04%	*S.aureus*	Subur	Ungu -> Kuning
	B2.1	BCP 0,04%	*E.coli*	Subur	Ungu -> Kuning
	B3.1	BCP 0,04%	*L.casei*	Subur	Ungu -> kuning
Uji	K1.1	Ekstrak kubis ungu	*S.aureus*	Tidak subur	Ungu -> Ungu
	K2.1	Ekstrak kubis ungu	*E.coli*	Tidak subur	Ungu -> Ungu
	K3.1	Ekstrak kubis ungu	*L.casei*	Tidak subur	Ungu -> Ungu

(Source: Suhartati, 2021)

The results of Suhartati's experiment (2021) in treatment 1 showed that purple cabbage extract was put into the media as an indicator to detect acids resulting from the fermentation of mannitol carbohydrates resulting in infertile bacterial growth so it cannot be used as an indicator which is characterized by no color change. Furthermore, 0.04% BCP control solution may function as an indicator. Purple cabbage extract that is not

inoculated with bacteria cannot function as an indicator because the ethanol contained in purple cabbage extract has antioxidant, antibacterial, and antifungal properties from phenol and flavonoid compounds, and can inhibit the growth of bacteria and yeast (Rakhmat et al., 2021).

Tabel 2. Hasil Uji Biokimia Perlakuan 2 Indikator diteteskan setelah selesai inkubasi

Uji Biokimia	Kode	Jenis indikator	Jenis bakteri	Pertumbuhan bakteri	Perubahan indikator
Kontrol	B1.2	BCP 0,04%	*S.aureus*	Subur	Tidak berwarna menjadi cincin kuning
	B2.2	BCP 0,04%	*E.coli*	Subur	Tidak berwarna menjadi cincin kuning
	B3.2	BCP 0,04%	*L. casei*	subur	Tidak berwarna menjadi cincin kuning
Uji	K1.2	Ekstrak kubis ungu	*S.aureus*	subur	Tidak berwarna menjadi cincin merah muda
	K2.2	Ekstrak kubis ungu	*E.coli*	subur	Tidak berwarna menjadi cincin merah muda
	K3.2	Ekstrak kubis ungu	*L. casei*	subur	Tidak berwarna menjadi cincin merah muda

(Source: Suhartati, 2021)

The results of the experiment (Suhartati, 2021) in treatment 2, where the purple cabbage extract was put into the media that had been inoculated by the bacteria showed that the bacteria were growing fast and there was no inhibition to the growth of the bacteria. The indicator of the ethanol extract of purple cabbage inoculum is said to work well because it can detect acids produced by the fermentation of carbohydrates, mannitol, and lactose. The formation of a pink ring on mannitol media cultured by S. aureus and E. coli bacteria is an indicator sign of purple cabbage being at a pH of around 5 (acidic) according to the experiment of Suerni et al. (2013). Acid is produced from the fermentation of the carbohydrate mannitol or lactose by these two bacteria (Pratiwi et al., 2021).

The purple cabbage indicator that was dropped at the end after the culture was incubated did not cause damage to the function of the indicator due to heating during media sterilization and the antimicrobial substances contained in the purple cabbage extract did not interfere with the growth of the bacteria being cultured. To assist in the interpretation of the results of the biochemical test of carbohydrate fermentation to identify pathogenic bacteria, the purple cabbage extract can be reacted after the inoculum has been incubated in the carbohydrate fermentation tube for the biochemical test.

▪ CONCLUSION

Based on the results of the discussion, it can be concluded that by reacting with the biochemical test media after the incubation phase, the ethanol extract of purple cabbage (Brassica oleracea L) can be used as a color indicator in carbohydrate fermentation tests on mannitol and lactose media. The experiment was carried out with 2 treatments, namely treatment 1, where the purple cabbage extract was added to the media composition before being inoculated with bacteria. In treatment 2, the purple cabbage extract indicator was added to the media that had been inoculated with bacteria.

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