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The Detection of Non-Formalin Food Additives Acid Compounds in Food Using Purple Sweet Potato (*Ipomoea batatas (L.) Lamb*) Anthocyanins

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Abstract
Formalin in food can be detected using anthocyanin pigments in purple sweet potatoes (*Ipomoea batatas (L.) Lamb*). Anthocyanins turn red or pink on contact with acidic formalin. Concerns arise about similar color changes with other acidic compounds. Thus, this study examines anthocyanin reactions with various acidic compounds—Food Additives Acids: acetic, lactic, citric, phosphoric, ascorbic, benzoic, and tartaric acid. This simple experiment involved 7 test samples and 1 control (formalin). Sample concentrations adhered to BPOM guidelines, and pH levels were measured. Purple sweet potatoes were mixed 1:1 with distilled water. 5 mL of each sample was combined with anthocyanin drops in separate test tubes (10 mL total volume). Observations tracked color change duration (735.40 seconds). pH was measured again post-observation**.** Results showed red coloration in lactic, phosphoric, and acetic acid samples—not always indicating formalin. pH changes after anthocyanin addition can quantitatively detect formalin and acid samples. This research has never been conducted before. Previous studies only focused on the detection of formalin acid compounds in food, not about acidic food additives.

Keywords: Formaldehyde, Acid, Anthocyanin

1. Introduction

Formalin is often misused as a food preservative, especially in finished and semi-finished processed foods. The use of formalin in food because of its ability to kill bacteria so as to make food durable. The use of formalin is very worrying when consumed in large quantities because it can cause health problems. Therefore, the presence of formalin in food is something that needs to be anticipated to prevent harm that enters the body. There are several studies that have found a way to detect formalin easily, quickly, and cheaply by utilizing anthocyanin pigments in purple sweet potato extract (*Ipomoea batatas (L.) Lamb*). Besides being able to be used as a natural dye, anthocyanins can also be used as formalin indicators in food (Syarif &; Yuni, 2016). Based on studies that have been conducted on the effectiveness of anthocyanin pigments to detect formalin in food, it shows the results that anthocyanin pigments undergo color change from purple to deep red as a result of the reaction of anthocyanins with strong acid formalin. However, these studies did not consider other acidic compounds that may be contained in foods. The final results that are observed qualitatively by only paying attention to the color changes that occur in anthocyanins are feared that the indications of color changes that occur are generalized only to formalin, even though it could be that other acid compounds also produce the same color, especially in strong acids. For this reason, this study will discuss the types of acidic compounds that may be contained in food. Furthermore, tests were carried out and observed reactions with anthocyanins in terms of color changes produced by considering the type of acid compound and pH owned. In addition, in this study will also be carried out quantitative observations of pH changes that occur in acid after reacting with anthocyanins.

2. Research Method

Time and Place

This research was conducted at the Chemistry Laboratory of the Faculty of Science and Technology, Universitas Terbuka, from April to July 2023.

Materials and Tools

Materials used include purple sweet potatoes harvested at 4 - 4.5 months of age, acetic acid, citric acid, and benzoic acid, formalin, tartric acid, lactic acid, phosphoric acid, ascorbic acid and distilled water. Tools used include knives, graters, filters, 5 mL Mohr pipettes, containers, 50 mL and 500 mL measuring cups, 15 mL test tubes, pH meters, timers, gloves, masks, and head coverings.

Research Stages

This research consists of 5 stages:

1. Adjustment of the concentration of food additive samples using BPOM standards;
2. pH measurement of samples whose concentration has been adjusted to the recommendations of the Food and Drug Supervisory Agency (BPOM);
3. Stage of preparation of the indicator solution of purple sweet potato;
4. Observation stage of acid discoloration after titrating with purple sweet potato solution;
5. pH measurement of the sample after adding anthocyanin droplets.

Research Procedure

1. Adjust the concentration of acid samples in accordance with the guidelines of the Food and Drug Supervisory Agency (BPOM). The concentration is adjusted according to the dose for the specific food type/product.
   a. Dissolve benzoic acid as much as 30 mg / 50 mL aquades;
   b. Dissolve acetic acid as much as 750 mg / 150 mL aquades;
   c. Dissolve citric acid as 50 mg/50 mL aquades;
   d. Dissolve tartric acid as much as 250 mg / 50 mL aquades;
   e. Dissolve lactic acid as much as 900 mg / 150 mL aquades;
   f. Dissolve phosphoric acid as much as 900 mg/100 mL aquades;
   g. Dissolve ascorbic acid as much as 50 mg/50 mL aquades;
   h. Dissolve formalin as much as 50 mg / 500 mL aquades;

i. Dissolve formalin as much as 100 mg / 500 mL aquades; (BPOM RI, 2013 ; BPOM RI, 2012, BPOM RI, 2019).
2. The pH of each sample is then measured using a pH meter, and the results are recorded. The same procedure is also performed for formalin;
3. The sweet potato is washed, peeled, washed again, finely grated, and then squeezed to obtain the extract. The sweet potato extract is then liquefied in aquades in a ratio of 1:1 (Setyawan &; Hanizar, 2021);
4. Pour 5 mL from each sample into a different test tube, then drip anthocyanin (indicator solution) until it reaches a volume of 10 mL. Observe color changes in 735.40 seconds (Setyawan &; Hanizar, 2021). Document the color changes in the samples;
5. Remeasure the pH of the sample using a calibrated pH meter to improve the accuracy of the measurement results;

Repeat this process three times, including observing the pH of the sample before and after adding anthocyanin droplets, and observing the color change of the sample after anthocyanin treatment.

3. Results and Discussions

Food is one of the primary needs of living things to maintain their lives (Mira, et al., 2019). Food can be consumed directly or indirectly by going through a series of processing first. Not infrequently, processed foods are given food additives (BTP), such as dyes, flavors, and preservatives. Food additives (BTP) are ingredients that are added intentionally to food in small quantities with the aim of improving appearance, taste, texture, increasing nutritional value and extending shelf life (Dhimas, 2010). The BTP group is allowed, especially acidic compounds that are often added to food are acetic acid, lactic acid, citric acid, phosphoric acid, ascorbic acid, benzoic acid, and tartric acid (Wahyudi, 2017; BPOM, 2018).

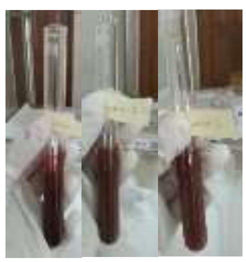
Before pH measurement is carried out, the concentration of acid BTP is adjusted first according to the recommended use of acid BTP in specific foods, referring to BPOM. The concentration of benzoic acid used is based on safe limits for coffee products, coffee substitutes, teas, herbal infusions, grain-based drinks and hot cereals, except chocolate, and packaged tea drinks. Acetic acid is used based on safe limits for baby and child food products in the growth period. Citric acid is used based on safe limits for dairy products and raw (pure) milk (excluding fresh milk). Tartric acid is used within safe limits for unsalted bakery products (e.g. bagels, ribbons, English muffins. Lactic acid is used based on safe limits for meat products, poultry, and pureed raw game meat. Phosphoric acid is used based on safe limits for uncured cheese products (raw cheese). Ascorbic acid is used based on safe limits for the needs of the body. Formalin 50 mg/500 mL aquades based on safe limits that the body can tolerate. Formalin 100 mg/500 mL aquades are not based on safe limits (BPOM RI, 2012 ; BPOM RI, 2013).

On the other hand, there are harmful BTP groups that are still often added to food, such as formalin, borax, and rhodamine B. Formalin is a dangerous BTP group, a strong acid compound that is often added as a preservative (Wahyudi, 2017; Saputra &; Ervina, 2019). Formalin is acidic because it contains formic acid due to formaldehyde oxidation (Nuhman &; Wilujeng, 2017). Formalin can cause poisoning because it quickly reacts with the mucous lining of the digestive tract and respiratory tract. Long-term use of formalin causes blood vessel failure, nervous system depression, kidney failure, hypotension, liver toxicity, and if exposed in large quantities can cause death (Yuliantini &; Winasih, 2019). Therefore, the presence of formalin needs to be detected to prevent the consequences caused by the consumption of formalin foods.

Qualitative Analysis

Qualitative analysis is carried out on the color changes that occur in anthocyanins after reacting with formalin and BTP acids. This will show a comparison of how the color change that occurs in formalin with the BTP acid that may be contained in food, so that people have wider considerations and options when detecting food using anthocyanins. The results of the formalin and BTP acid reactions are shown in the following figure arranged based on the sequence of repetitions 1, 2, and 3.

Picture 1. Tartric Acid



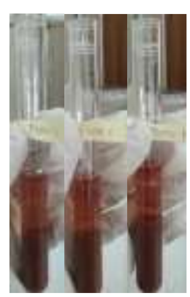
Picture 2. Lactic Acid

Picture 3. Formalin (1) g

Picture 4. Formalin (0,5 g)

Picture 5. Phosphoric Acid

Picture 6. Acetic Acid



Picture 7. Citric Acid

Picture 8. Ascorbic Acid

Picture 9. Benzoic Acid

The images are successively samples of tartric acid, lactic acid, formalin (1g), formalin (0.5 g), phosphoric acid, acetic acid, citric acid, ascorbic acid, and benzoic acid. The results of qualitative observations showed that there was a significant difference between the color change in formalin and other acid BTP. The following table presents the color change of anthocyanins after contact with BTP acid and formalin along with the initial pH range of BTP acid and formalin along with the precipitate formed.

Table 1.1. First pH Range and Color Changing's Samples

Food Additives Acids	First pH Range	Color Changing	Precipitate
Tartric Acid	1,6 – 2,4	Brownies red (red dominantly)	80 %
Lactic Acid	1,82	Purplish red (red dominantly)	85 – 90 %
Formalin (1 g)	7,28 – 7,7	Deep purple (towards black)	0 %
Formalin (0,5 g)	7,17 – 7,38	Deep purple (towards black)	20 %
Phosphoric Acid	0,62 – 2,17	Bright red	50 %
Acetic Acid	2,7 – 3,51	Brownies red (red dominantly)	70 %
Citric Acid	2,48 – 2,59	Deep brown	10 %
Ascorbic Acid	3,47 – 3,8	Deep purplish red (purple dominantly)	0 – 5 %
Benzoic Acid	6,76 – 7,32	Deep purplish red (purple dominantly)	0 %

The anthocyanin color change shown in formalin is a deep purple color (towards black). This shows a negative relationship with the results of research conducted by (Saati et al, 2016; Rochyani et al, 2017), showed that anthocyanins that react with formalin produce increasingly concentrated red or pink discoloration. In addition, research conducted by (Saputra &; Ervina, 2019) regarding the detection of formalin tofu showed that anthocyanins changed color to red or pink. Similar results were shown in the salted fish formalin test study, it was proven that formalin salted fish samples gave the same color, namely red and pink (Setyawan &; Hanizar, 2021). While the test results of formalin milkfish give a purplish red color to anthocyanins. These studies show that the change in anthocyanin color to red or pink occurs only in formalin. The results of our analysis show that the red color does not necessarily indicate formalin, but can also be acidic BTP compounds (lactic acid, phosphoric acid, and acetic acid). The resulting deep purple color (towards black) is due to the difference in the concentration of formalin used. The formalin sample (0.5 g) has been pre-dissolved with aquades to adjust the concentration of formalin tolerated by the body. The formalin sample (1 g) showed similar results with differences in precipitate concentration. So, if the community conducts a formalin analysis test and changes to deep purple (towards black), then the formalin levels contained are still within the body's tolerance limit.

Red or predominantly red color is found in acid samples that have a pH (0.62 – 3.51). This is in accordance with the statement stated by (Saati et al, 2016; Rochyani et al, 2017) that anthocyanins will be red or pink if they react with acids. Anthocyanins are pigments that produce purple, pink, peacock red, blue, and red colors on leaves, flowers, and fruits (Nasution, 2016). Anthocyanins are amphoteric, having the ability to react with both acidic and alkaline compounds (Saati dkk, 2016; Rochyani dkk, 2017).

Qualitative observations made are prone to causing errors because of the different perspectives of each person on different colors. For this reason, quantitative observations are also carried out to obtain more objective data in the form of pH changes in formalin and acid samples after reacting with anthocyanins.

Quantitative Observation

The results of quantitative observations of sample pH after measuring using a pH meter with 3 repetitions are presented in table 1.2.

Table 1.2. Initial Sample pH

	Formalin (0,5 g)	Formalin (1 g)	Asam Laktat	Asam Asetat	Asam Askorbat	Asam Benzoat	Asam Sitrat	Asam Tartrat	Asam Fosfat
Ulangan 1	7,38	7,28	1,82	2,7	3,8	6,76	2,59	1,6	0,62
Ulangan 2	7,28	7,59	1,82	3,51	3,47	7,32	2,48	2,4	2,09
Ulangan 3	7,17	7,7	1,82	3,51	3,47	7,13	2,48	1,6	2,17
Rata-Rata	7,28	7,52	1,82	3,24	3,58	7,07	2,52	1,87	1,63

The measurement results between repetitions 1,2, and 3 showed a difference, but not significant. This difference can be caused by the value of accuracy of the pH meter. The pH measured sample was then given anthocyanin droplets. Re-observation is carried out by measuring pH using a calibrated pH meter. The measurement results are presented in table 1.3.

Table 1.3. pH of Samples + anthocyanin

	Formalin (0,5 g)	Formalin (1 g)	Asam Laktat	Asam Asetat	Asam Askorbat	Asam Benzoat	Asam Sitrat	Asam Tartrat	Asam Fosfat
Ulangan 1	7,28	7,37	4,34	3,91	7,13	6,86	5,66	3,8	3,04
Ulangan 2	6,7	6,7	4,58	4,6	6,86	6,76	5,33	3,91	2,59
Ulangan 3	7,04	7,13	5,1	4,01	7,11	6,76	5,59	4,8	3,23
Rata-Rata	7,01	7,07	4,67	4,17	7,03	6,79	5,53	4,17	2,95

Table 1.4. Average pH Differences

Formalin (0,5 g)	Formalin (1 g)	Asam Laktat	Asam Asetat	Asam Askorbat	Asam Benzoat	Asam Sitrat	Asam Tartrat	Asam Fosfat
0,27	0,46	-2,85	-0,93	-3,45	0,28	-3,01	-2,3	-1,33

There was a significant change in the pH of the sample before and after anthocyanin was given. Ascorbic, lactic acid and citric acid become more alkaline after anthocyanin administration, with each an average pH difference of -3.45 ; -2,85 ; and -3.01. Other samples, such as acetic acid, tartric acid, and phosphate also became more alkaline but did not have a significant difference with an average difference of -0.93 each; -1,33 ; and -2.30. While formalin and benzoic acid become more acidic after being given anthocyanins. The difference in pH in each sample can be caused by the level of acid solubility in the water. The difference in pH changes in each sample after being given anthocyanins can be caused by the concentration of acid, air temperature, water temperature, and the level of acid solubility in the anthocyanin.

Table 1.5. ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	58,33700741	8	7,29212593	68,7168086	6,52922E-12	2,510157895
Within Groups	1,910133333	18	0,10611852			
Total	60,24714074	26				

The results of ANOVA test analysis showed a significant difference between treatments ($p < 0.05$) as shown in table 1.4. Therefore, researchers conducted Duncan's follow-up test.

Table 1.6. DUNCAN

			Subset for alpha = 0.05			
PERLAKUAN		**N**	**1**	**2**	**3**	**4**
Duncan[a]	Asam Fosfat	3	2,9533			
	Asam Tartrat	3		4,1700		
	Asam Asetat	3		4,1733		
	Asam Laktat	3		4,6733		
	Asam Sitrat	3			5,5267	
	Asam Benzoat	3				6,7933
	Formalin (0,5 g)	3				7,0067
	Asam Askorbat	3				7,0333
	Formalin (1 g)	3				7,0667
	Sig.		1,000	0,089	1,000	0,358

Duncan's further test results showed that the pH in each treatment varied. Formalin (1 g), ascorbic acid, formalin (0.5 g), and benzoic acid have no different pH. The pH of citric acid is different from other acids. Phosphoric acid has a different pH from other acids, while lactic acid, acetic acid, and tartric acid have no different pH. The results of such an analysis show that there is no noticeable difference between the pH of formalin with ascorbic acid, and benzoic acid, so that pH measurements cannot be used as indicators to detect formalin with concentrations of 0.5 g / 500 mL and 1 g / 500 mL.

4. Conclusions

A change in anthocyanin color from purple to red does not necessarily indicate formalin. There are several other acids that give the same color change, such as lactic acid, phosphoric acid, and acetic acid. Formalin with the body's tolerance limit has a color change to deep purple (towards black). There is a change in the pH of the sample after anthocyanin administration, such as formalin and benzoic acid, while other samples become more alkaline. Such changes are due to the degree of solubility of acids in anthocyanin.

Advice

For future research, it is recommended to test BTP for other acids that may be present in food. It is recommended to use food or beverage samples that do not have standards, such as school snacks and traditional market snacks. Analyze the acid content of the reaction material with anthocyanins. Routine calibration of measuring devices such as pH meters must be performed to maintain the accuracy of analysis results. The qualitative analysis of color changes in anthocyanins can be improved by quantitative analysis, by measuring the wavelength of a given color. Deposits formed on acid after anthocyanin treatment need to be analyzed regarding the cause, process, and effect on the color and pH of the sample.

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