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Prevention of Carotenoids as Natural Colorant in Kabocha Squash's Concentrate by the Addition of Magnesium Carbonate

Running Title: Fixation of Carotenoid by $MgCO_3$

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Abstract Carotenoids as hydrophobic pigments can be degraded due to acidic conditions in the hydrophilic system during the extraction and encapsulation processes of the colorant powder derived from kabocha squash. This research was conducted to determine the effect of using magnesium carbonate ($MgCO_3$) as a fixation agent to maintain carotenoid stability in the process of making natural colorant powder from kabocha squash. The concentrated extract of kabocha was added with several concentrations of $MgCO_3$ (0, 1, 1.5, 2, 2.5, and 3%) of the total extract. The acidity condition of kabocha pumpkin extract was observed periodically to prove the existence of acids. Changes in pH were also observed in the treated solution. The total content of carotenoids in the resulting colorant powder was also observed to determine the effectiveness of the fixation agent. Observations of color changes were also carried out to see the appearance of the color of the resulting colorant powder. This study confirmed that the hydrophilic system in the carotenoid extraction process results in the release of acids which lowers the pH of the solution. The optimal addition of $MgCO_3$ was obtained at a concentration of 2%, where excess fixation agents could also reduce existing carotenoid compounds. The pH was crucial for maintaining the carotenoid content in the hydrophilic system derived from plant extracts. In the production process of natural colorant powder from kabocha squash, pH 9.2 was suitable for producing the highest carotenoid content with acceptable colour characteristics. This study underlined the importance of a fixing agent ($MgCO_3$) to keep the carotenoids in the kabocha dye powder stable.

Keywords Fixation, Industrialization, Natural Pigment, in Polar-Solution, Stability

1. Introduction

Functional plant concentrates with high carotenoid content offer attractive color options for both natural and artificial color additives as consumer expectations shift to more natural ingredients [1]. However, the development of natural dye products has problems related to the character of natural components which are unstable in many conditions [2]. The complex matrix in a network of plant components and the oxidative susceptibility and degradation of carotenoids as active ingredients present the main unique problems in replacing artificial colors with plant concentrated colorants. Carotenoids are lipophilic pigments, neutral, and tend unstabilized in hydrophilic environments. Plant tissue is known to have fairly high water and organic acid content that can affect the stability of carotenoids [3]. Theoretically, the presence of ionized acid can cause the breakdown, dehydration, or isomerization of carotenoids. Cyclic carotenoids with 5,6-epoxides, such as violaxanthin and neoxanthin, are easily subjected to rearrangement to produce the proper 5,8-epoxide [4]. This phenomenon can affect the color quality of carotenoids due to changes in the chromophore structure so that the specific color of carotenoids disappears.

Therefore, efforts must be conducted to stabilize carotenoids in natural colorants derived from plants. Information needs to be reported regarding efforts to maintain the stability of carotenoids in natural colorants produced from the pulverization process of plant concentrates that are high in carotenoids since, at the pulverization process, all compounds would be extracted, including the substance that interrupts the stability of carotenoids such as organic acids. The production of natural colorant by powdering liquid concentrates is safe because it does not use any chemicals, but this action is very susceptible to the oxidation process of carotenoids. In this research, the preparation of natural coloring powder containing carotenoids from kabocha pumpkin will be carried out. Kabocha pumpkin (*Cucurbita maxima*) Duchesne is a very potent source of carotenoid. Kabocha has a higher carotenoid content than local pumpkin, which is suitable to be processed as a food colorant [5]. The use of fixation agents in the process of making carotenoid natural colorant powder from kabocha pumpkin was focused on in this study. Several parameters clarified the effectiveness of the fixation agent in maintaining the acid condition.

Fixation agents such as magnesium carbonate ($MgCO_3$) are known to neutralize the carotenoid in the hydrophilic system from the acids released during the tissue disintegration process [4]. The prevention of carotenoid breakdown by fixation will stabilize the carotenoid in hydrophilic condition during the extraction and encapsulation process of kabocha concentrate which extended the self-life of produced colorant. This condition would certainly be highly expected from an industrial perspective. Thus, by optimizing the use of $MgCO_3$ to stabilize carotenoids, basic information regarding developing natural colorants containing more stable carotenoids during manufacturing and storage processes would be provided. The findings from this study will be used as guidance for the future industrialization process of natural carotenoid colorants.

2. Materials and Methods

2.1. Study Area

The research was conducted in the period of April 2022 and March 2023. The research was conducted at the Laboratory of Phytonutrients, Department of Agricultural Product Technology and Laboratory of Biotechnology, Faculty of Animal Science, Andalas University.

2.2. Sample Preparations

The sample (approx. 1 Kg) that had been weighed was then washed and cut into small pieces. The pieces were crushed using a food blender (Panasonic MK-F800SSR, Jakarta, Indonesia). The crushed sample was filtered, and the dregs were separated to obtain the filtrate (concentrate). According to the treatment, Kabocha pumpkin concentrate was added with 5% maltodextrin as an encapsulator and magnesium carbonate ($MgCO_3$) as a stabilizer. The addition of $MgCO_3$ was conducted as 0, 1, 1.5, 2, 2.5, and 3% from the total concentrate. The drying process was carried out using the tray dryer with a food dehydrator (FDH-10, Bekasi, Indonesia) at 60℃ for 8 hours. After drying, grinding and sifting were carried out to produce a natural kabocha squash colorant powder. The produced powder was then analyzed for color, pH, and total carotenoids.

2.3. pH Analysis

pH analysis was carried out for the concentrate solution of kabocha and treated (encapsulation + fixation) solution of colorant. For the solution, the pH was measured periodically for three hours. The pH was measured with a pH meter (Metler Toledo F20 Standard, Albstadt GmbH, Germany). The pH meter was calibrated with buffer stock solution (pH 4.01; 7.00, and 9.21) prior to measurement [6-7].

2.4. Colour Analysis

The color measurement was performed using a colorimeter (Hunterlab color Flex EZ Spectrophotometer). The sample was inserted into the container until the sample surface was completely covered. The information for each variable (L*, a*, and b*) was then recorded [8-10]. The following equation was used to indicate the colour direction of the sample.

$$^{\circ}\text{Hue} = 360 + (\tan - 1 \ \ b/a).$$

where: °Hue = colour direction, a; represents the direction of color between red and green (negative values denote green, while positive values denote red), and b; represents the direction of color between yellow and blue. (negative values indicate blue and positive values indicate yellow).

2.5. Analysis of Total Carotenoids by Spectrophotometer

In a 50 mL volumetric flask, 100 mg of sample and 50 mL of hexane were combined, agitated until perfectly homogenous, and then diluted 10 times. In the test tube, 1 mL of the solution was added to 9 mL of hexane. At a wavelength of 446 nm, the solution's absorbance (A) was measured. This measurement was conducted with a Shimadzu UV-1800[11]. The following equation was used to determine the total amount of carotenoids in the samples:

$$\text{Carotenoids content } (\mu g/g) = \frac{25 \text{ x A x } 383}{100 \text{ x sample weight (g)}} \text{ x Dilution factor} \quad (10)$$

Note: A is the absorbance at 446 nm; 383 is the carotene diffusion coefficient; V is the hexane value (mL), and W is the sample weight (g).

2.6. Statistical Analysis

Utilize the SPSS package application version 11.5 for statistical analysis (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) and Duncan's multiple ranges posthoc test were used to evaluate the data. Significance level alpha=0.05 was used to determine the significance of differences.

3. Results and Discussion

Figure 1 shows the change in pH of the kabocha squash concentrate solution before being treated into powder. The pH of the solution was measured for 3 hours with observation intervals every 30 minutes. This observation was done because, practically, there will be a waiting time in the concentrated solution before the powdering process. At this time, the release of organic acids in the concentrate can affect the stability of the carotenoid compounds. From the results of the pH analysis, it can be seen that there was a gradual decrement in the pH of the concentrate solution for 3 hours. This data has clarified that organic acids could quickly appear in the kabocha squash concentrate solution. Many reports have stated the presence of organic acids in plant tissues. At least eight types of organic acids have been confirmed to be present in plant tissues, namely glyoxylic acid, tartaric acid, glycolic acid, malic acid, acetic acid, citric acid, and succinic acid. These organic acids are essential in helping a plant's photosynthesis process [3,12,13]. Therefore, the manufacture of carotenoid powder dyes by utilizing water-based concentrate from a plant has challenges due to the presence of these organic acids. Thus, the application of $MgCO_3$ can be used as a solution.

Figure 2 indicated the pH value of the kabocha squash concentrate solution in which maltodextrin was added as an encapsulator and $MgCO_3$ as a fixator. It can be seen that there was an increase in pH due to maltodextrin and $MgCO_3$ treatment. In this study, the preparation process and the addition of maltodextrin and $MgCO_3$ homogenization took up to 1 hour. This condition cannot be avoided and becomes a critical waiting time that will be found in the pulverization process. The pH range obtained in the mixed solution is between 8.8 and 9.5, where this condition was already in an alkaline condition in spite of without $MgCO_3$ addition. Nurhadi et al [14] indicated that maltodextrin has slightly alkaline conditions which means it also induces the increment of pH of the solution. Some literature also reports that extreme alkaline conditions are unsuitable for a carotenoid compound's stability [15-16]. This condition might not be unwanted as well. Therefore, observing the optimum concentration of carotenoids in the produced colorant was necessary.

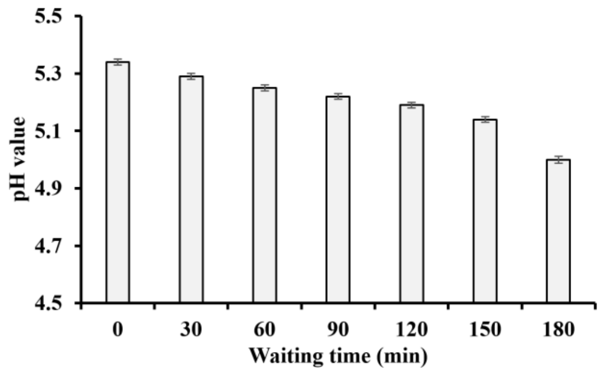

Figure 1. The Changes in pH in the concentrate solution of kabocha squash

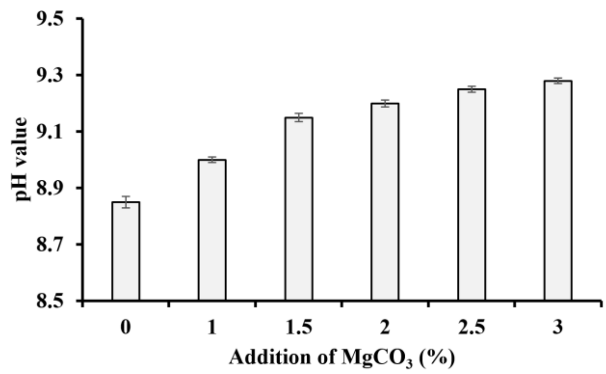

Figure 2. The pH of the solution during the encapsulation process containing $MgCO_3$

Figure 3 shows the total carotenoid content in kabocha squash colorant powder made from the drying process of concentrate solution with the addition of various concentrations of $MgCO_3$. The lowest carotenoid content was found in the treatment without adding $MgCO_3$. The presence of carotenoids increased with the addition of $MgCO_3$, but when $MgCO_3$ was added above 2%, carotenoid levels decreased. The pattern of carotenoid content in the resulting powder colorant was fascinating. A hypothesis could be proposed: at the addition of $MgCO_3$ below 2%, carotenoid degradation might occur during the waiting time due to acidic conditions, while in the treatment of $MgCO_3$ above 2%, carotenoid degradation occurred during encapsulation where the pH conditions were very high. The concentration of $MgCO_3$ at 2 % was resulted in the pH condition at 9.2. In this condition, the presence of acid and base ions had a minimum effect on carotenoid compounds (Fig. 4).

The finding in this study showed that the encapsulation process by maltodextrin without the fixation process by $MgCO_3$ cannot maintain the carotenoid content because maltodextrin cannot neutralize the pH of the existing solution system. The addition of $MgCO_3$ with a particular concentration was very important to get the optimum carotenoid value in a desired natural carotenoid colorant. However, in this study, the concentration of $MgCO_3$ addition was not optimized due to the large range of treatments therefore, the optimation should be conducted further by using response surface methodology.

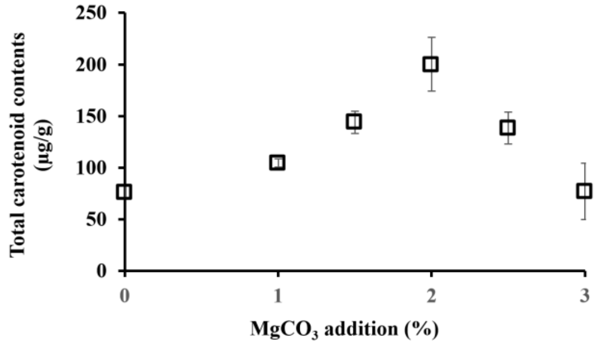

Figure 3. The pattern of total carotenoids in produced colorant powder treated by $MgCO_3$

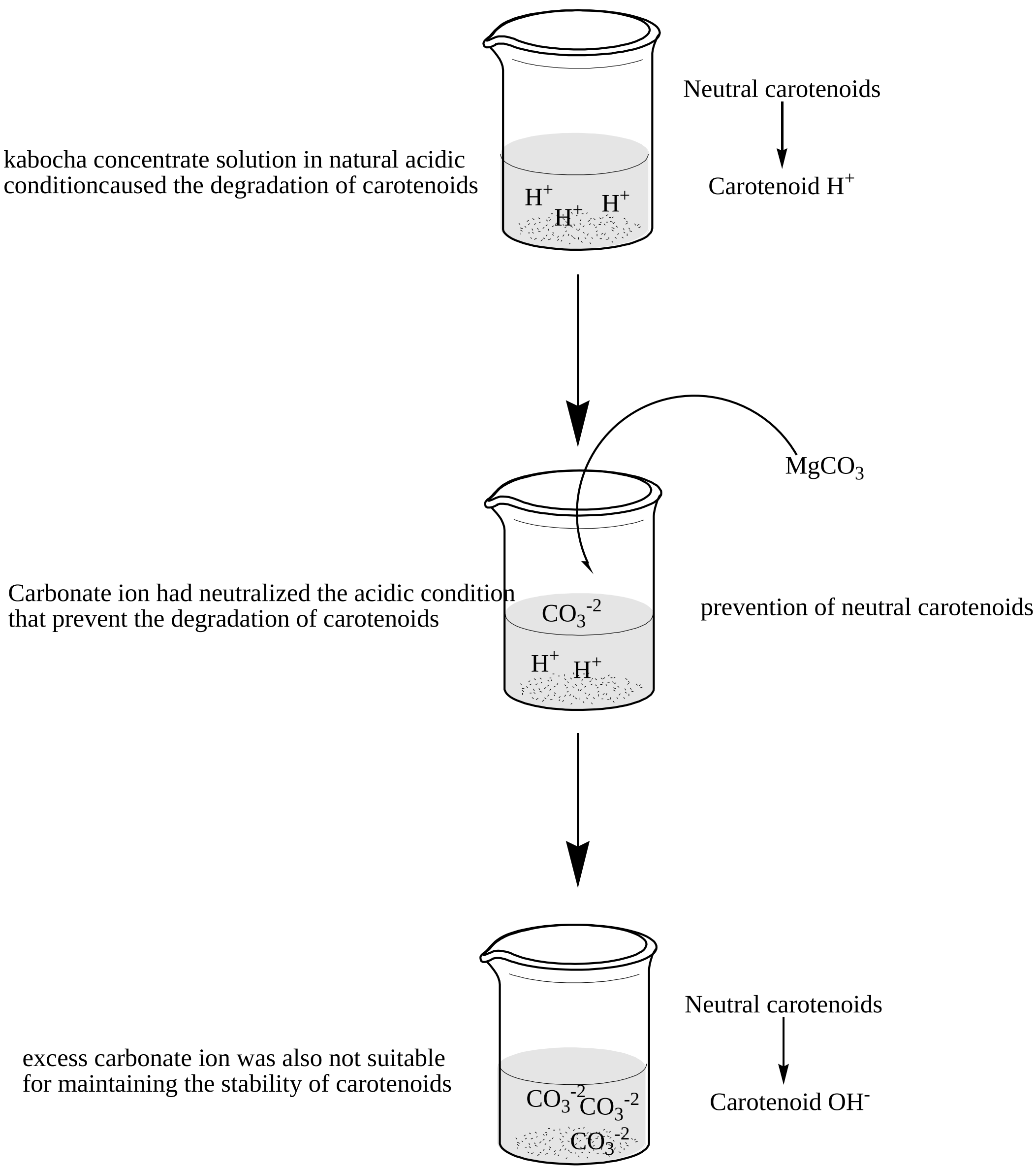

Figure 4. Illustration of $MgCO_3$ mechanism on prevention of carotenoids in water-based plants concentrate extracts

Table 1. The colour characteristic of produced colorant powder

$MgCO_3$ Treatments	L	a*	b*	˚Hue	Colour criteria
0%	57.50 + 0.53	29.17 + 0.97	59.84 + 0.57	64.01 + 0.93	Yellow –red
1%	71.01 + 0.20	20.13 + 0.11	53.76 + 0.60	69.82 + 0.26	Yellow –red
1.5%	78.15 + 0.01	14.83 + 0.25	52.05 + 0.86	75.28 +0.46	Yellow –red
2%	85.89 + 0.19	12.62 + 0.82	49.73 + 0.10	76.372+ 0.23	Yellow –red
2.5%	85.93 + 0.70	6.56 + 0.72	35.05 + 0.52	76.74+ 1.03	Yellow –red
3%	89.77 + 0.29	6.07 + 0.97	24.79 + 0.33	77.73+ 1.68	Yellow –red

Note: The values of lightness (L) and hue/saturation (a= red/green coordinate, b= yellow/blue coordinate) in the L, a, and b color spaces, which were measured using a spectrophotometry method, were used to describe the color characteristics of the sample.

Table 1 indicates the colour characteristics of produced colorant powder from kabocha squash. The lightness of the product was increased according to the addition of $MgCO_3$. This increment was due to the addition of $MgCO_3$, which has a white color. According to Gomes and Oliveira [17], the whiter fillers added, the brighter the color of the powder product. From the perspective of colour direction, indicated by the ˚Hue value, a similar colour direction was observed with a hue value in the range of 64-77, red-yellow (orange), which indicates that the addition of $MgCO_3$ increased the ˚Hue value. The changes of color might be caused mainly due to the color interruption by the addition of $MgCO_3$. Although the color interruption from $MgCO_3$ might be dominant compared to the carotenoid degradation, the information on the carotenoid content has shown the critical role of $MgCO_3$ in maintaining carotenoid stability. The use of $MgCO_3$ as a fixator has been widely developed for chlorophyll compound stabilizers [18-20] but none reported it for carotenoid compounds. Therefore, the results of this study can be a reference for other hydrophobic pigments. Further information needs to be explored from this study, whether $MgCO_3$ can maintain the stability of carotenoids in the colorant powder during storage. Powder storage conditions tend to be affected by the oxidation process by air which is quite an interesting topic.

Similar to preventing chlorophyll degradation in an acidic environment, using $MgCO_3$ in carotenoid pigments can also avoid the decomposition of carotenoid compounds in an acidic environment. Carotenoids can be damaged by various factors, including light, especially ultraviolet (UV) light, which can cause damage to carotenoids. This condition is because UV light can break down the molecular structure of carotenoids. Therefore, fruits and vegetables rich in carotenoids should be stored in a dark place. High temperatures can also cause damage to carotenoids. This is because high temperatures can accelerate the oxidation process of carotenoids. Therefore, fruits and vegetables rich in carotenoids should be cooked at a reasonable temperature. Acids can also cause damage to carotenoids. This is because acid can hydrolyze carotenoids, damaging the molecular structure. Therefore, fruits and vegetables rich in carotenoids should not be prepared with acidic ingredients like vinegar or citrus. Oxygen can also cause damage to carotenoids. This is because oxygen can oxidize carotenoids, damaging the molecular structure. Therefore, fruits and vegetables rich in carotenoids should be processed and consumed immediately.

In making coloring powder from kabocha juice, all factors that are sources of damage to carotenoid pigments are very likely to be present. The acid formation was a damaging factor that might be present in the sample because, in fruit and vegetables, the presence of organic acids cannot be prevented. Moreover, water, which was used as a solvent in the extraction process, quickly dissolved organic acids. The use of water solvents was to increase efficiency and food safety because they do not use expensive organic solvents, which may have toxicity effects. Therefore, it is essential to ensure that acidic conditions do not affect the stability of carotenoids if you want to make coloring products from kabocha pumpkin. The results obtained from this research can be a reference for preventing carotenoid degradation by adding $MgCO_3$ to the squash that will be powdered.

4. Conclusions

This study confirmed the importance of using a fixing agent ($MgCO_3$) to stabilize carotenoids in the dye powder from the kabocha squash. The use of approximately 2% $MgCO_3$ resulted in a pH condition of 9.2 as the suitable treatment to prevent the stability of carotenoids. However, due to the wide range of concentrations of $MgCO_3$, further optimization needs to be carried out. This fixator can also be developed to prevent damage to other natural pigments, especially those that are hydrophobic and susceptible to acidic conditions and are present in the hydrophilic system of a plant water-based extract.

Author's Contributions

Daimon Syukri: wrote the manuscript; Aisman; Statistical Analysis and financial administrator as well as research leader; Skunda Diliarosta: Responsible revision of the manuscript; Aulia Azhar: Research administrator.

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Conflict of Interest

The authors have no conflict of interest.

Data Availability Statement

The data that support the findings of this study are openly available.

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