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EFFECT OF CALCIUM CHLORIDE CONCENTRATION ON IMPROVING THE QUALITY OF RESTRUCTURIZED STRAWBERRY FRUIT (*Fragaria x ananassa*)

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ABSTRACT

Strawberry is a very popular fruit due to their bright red color and aroma. sweet and slightly sour taste and nutritional value, especially vitamin C. Strawberries also contain anthocyanins, a reddish-purple pigment with high antioxidant activity. Nevertheless, they have a relatively short shelf life and are easily damaged because of their soft texture. They are usually processed into jam and other products that use high temperatures, which can damage nutritional compounds, including anthocyanins. Restructuring is an alternative method that can be used to extend the shelf life of strawberries without heat, thereby avoiding damage to the nutritional value and antioxidant activity. This study aimed to determine the effect of various Alginate and $CaCl_2$ concentrations (0, 0.1, 0.2, 0.3, 0.4, and 0.5%) on the quality of restructurized strawberries. The anthocyanin content was determined using the pH differential method; the texture was determined using a simple test to distinguish differences between treatments; the organoleptic test used hedonic methods with 30 panelists; and the shelf life tests were used to keep the real product until the quality decreased. Preliminary experiments showed that 2% alginate was optimal. The second step showed that 0.4% $CaCl_2$ was optimal.

Keywords: alginate, anthocyanin, $CaCl_2$, restructurized, strawberry

INTRODUCTION

Strawberries are highly popular in Indonesia, despite not being native to the region. They are widely cultivated across Indonesia and consumed by people of all ages, both fresh and processed into various products such as jam, syrup, dodol (traditional confectionery), preserved fruit, juice, and ice cream (Zaimah et al., 2013; Susianti, 2015)

Processing strawberries has been widely practiced for many years to prolong the shelf life of food products, including jams, preserved fruit, syrups, and 'dodol'(sweet treat). All of these processing methods require heating, which can destroy vitamin C and anthocyanins that are susceptible to heat, thus reducing their nutritional value. In addition to heating, processed jams, syrups, and dodol require the addition of significant amounts of sugar, making them unsuitable for diabetics. Another method to extend the shelf life of strawberries is through restructuring.

Restructuring is a physical and chemical process that reshapes the structure of food using a binder into a 'jelly like product' that meets consumer desires in terms of shape, size, texture, appearance, and taste. One example of a gel system as a chemically induced (non-thermal) binder is a combination of alginate with calcium ions. Fruit restructuring can be performed using sodium alginate with calcium lactate or calcium chloride (Raharjo and Utama, 2002). The restructurized method has been applied to jambolan fruit (Herawati et al., 2016) and strawberry fruit (Lestario, 2022). However, these studies achieved limited shelf life extension (3-4 days in a refrigerator), indicating the need for optimization of alginate and calcium chloride concentrations.

This study aimed to (1) determine the optimal concentrations of sodium alginate and $CaCl_2$ for strawberry restructuring and (2) evaluate the effects of these concentrations on anthocyanin retention,

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texture, and sensory acceptability of the restructurized product.

MATERIAL AND METHODS

Material specifications

The materials used in this research were sodium alginate, calcium chloride ($CaCl_2$), HCl, sodium acetate, KCl, ethanol (Merck, Germany), and granulated sugar (Gulaku). Strawberries (mature, red) were purchased fresh from a traditional market in the Getasan district on the Kopeng-Salatiga road, brought to the laboratory, cleaned from dirt (dust, sand, and leaves), washed under tap water, decanted for approximately 5 min, and then processed for restructurized fruit.

The tools include: analytical balance (OHAUS Pioneer PA214); mixer (Cosmos - 1279); UV-VIS spectrophotometer (Genesys, USA); cuvette; oven; desiccator; cutting board and knives.

Experimental Design and Analysis

The experiment was arranged in a Completely Randomized Design with six levels of calcium chloride concentration (0.0, 0.1, 0.2, 0.3, 0.4, and 0.5% w/v) as the independent variable, while sodium alginate concentration was fixed at 2% w/v based on preliminary optimization. Each treatment was replicated thrice, resulting in 18 (6 × 3) experimental units. Data on anthocyanin content, texture properties, and sensory attributes were subjected to analysis of variance (ANOVA) using SPSS version 25.0, IBM Corp., USA, followed by the Least Significant Difference (LSD) test at 95% confidence level ($\alpha = 0.05$).

Research Procedure

Preparation of Restructurized Strawberry

Restructurized strawberry was prepared following the method of Lestario et al. (2020) with modifications. Fresh strawberries were washed, hulved, and homogenized into pulp. A total of 100 g strawberry fruit pulp was mixed with 2% (w/w) sodium alginate and homogenized using a blender for 5 min. Granulated sugar (7 g) was added and mixed for 1 min. Subsequently, calcium chloride solution at varying concentrations (0.0, 0.1, 0.2, 0.3, 0.4, and 0.5% w/v) was added and homogenized for 2 min. The mixture was transferred to containers and stored at 4°C for 2 h to allow complete gel formation.

Determination of Texture

In the absence of a texture analyzer, the texture was evaluated using a penetration test with a standardized toothpick. A wooden toothpick was pressed vertically into the product at three different locations using consistent manual pressure. Resistance to penetration was assessed and rated on a 5-point scale as follows: 1 = very soft and watery, 2 = soft, 3 = moderately firm, 4 = firm, and 5 = very firm. Three replicate measurements were performed for each sample. Although semi-quantitative, this method provided comparative data among treatments. Future studies should use instrumental texture analysis for more objective measurements.

Anthocyanin Content Determination

The total anthocyanin content was measured using the pH differential method (Cheng and Breen, 1991). Samples (2 g) were macerated with 60 mL methanol-HCl 1% in a refrigerator (10 °C) overnight, filtered, and the waste was re-extracted twice with the same solution (2 × 20 mL). The filtrate was collected in a 100 mL flask, and the same solution was added to the calibration mark. Several milliliters of the anthocyanin extract were diluted with pH 1.0 buffer (0.025 M KCl) and pH 4.5 buffer (0.4 M sodium acetate) for comparison. The absorbance was measured at 510 and 700 nm using a UV-Vis spectrophotometer. Anthocyanin concentration was calculated using the Lambert-Beer equation:

$$A = \varepsilon\, b\, c;$$

where:

A = $[(A510\text{-}A700)\,pH1 - (A510 - A700)\,pH4{,}5]$
ε = molar extinction coefficient of cyanidin 3-glucoside (29,600 L mol^{-1} cm^{-1})
b = path length (1 cm)
c = anthocyanin concentration (mol/L).

The results were expressed as mg cyanidin-3-glucoside equivalents per 100 g of sample.

Determination of Water Content (Oven Method)

The water or moisture content of the 'restructurized fruit' was determined by drying an empty cup in an oven at 105 °C overnight and then cooling it in a desiccator. A 2 g sample of 'Restructurized Fruit' was placed in the cup and dried in an oven at 105 °C for 4– 6 h, cooled in a desiccator, and weighed again until constant. The water content was calculated using the following formula:

$$\text{Water content (\% wb)} = \frac{\text{Water weight}}{\text{Initial weight}} \text{ x } 100\%$$

Organoleptic Test

Organoleptic tests, including color, aroma, taste, texture, and overall, were conducted according to Soekarto (1985). This was done using a hedonic test with 30 panelists. The scale used was as follows: 1 = dislike very much, 2 = dislike, 3 = somewhat like, 4 = like, and 5 = like very much.

Shelf Life Test

The shelf life of the restructurized strawberries was determined by storing the product in a refrigerator and observing its quality (color, aroma, and flavor) (Yusuf, 2023). The samples were checked for mold every 3 days until the quality decreased (different color, aroma, and flavor). Once the sensory attributes became abnormal or any mold was observed, the observation stopped, and the shelf life was determined. Future research will observe the samples every day to obtain a more accurate shelf life.

RESULTS AND DISCUSSION

Texture

The texture o f the restructurized fruit with the addition of 2% sodium alginate and various concentrations of calcium chloride (0.0, 0.1, 0.2, 0.3, 0.4, and 0.5) is shown in Table 1.

Table 1. Effect of calcium chloride concentration on texture, anhocyanin content and water content of restructurized strawberry fruit products

$CaCl_2$ (%)	Texture	Anthocyanin Content (mg/100 g)	Water Content (%)
0.0	1.00 ± 0.00^{a}	27.52 ± 0.98^{a}	80.12 ± 0.01^{e}
0.1	2.17 ± 0.29^{bc}	26.48 ± 0.99^{a}	71.30 ± 0.52^{c}
0.2	2.67 ± 0.29^{bc}	24.23 ± 6.30^{a}	76.28 ± 0.04^{d}
0.3	3.00 ± 1.00^{c}	24.18 ± 1.90^{a}	69.20 ± 0.23^{b}
0.4	3.17 ± 0.76^{c}	22.81 ± 4.69^{a}	64.96 ± 0.11^{a}
0.5	1.92 ± 0.38^{ab}	22.11 ± 1.38^{a}	76.76 ± 0.71^{d}

Notes: Data (mean± standard deviation) were obtained from three replicates. Data were analyzed using ANOVA. Data within the same column followed by different letters indicate a significant difference (LSD test, $p<0.05$).

The Results showed that the higher the concentration of $CaCl_2$, the firmner (harder) the texture, it can be said that increasing concentration of $CaCl_2$ can improve the texture or make the restructurized fruit harder, it was good because it can increase the shelf life of the restructurized product.

Alginate and $CaCl_2$ formed a crosslinking bond, where the Ca^{2+} ion binds two adjacent alginate molecules to form an 'egg box', so that the sodium alginate, which was originally liquid (soluble in water), formed a solid gel structure. Based on the bond type, more Sodium Alginate is needed than $CaCl_2$ because Ca^{2+} is only needed as a connector between two alginates. Thus, the more $CaCl_2$ is added, the more cross-links (egg boxes) are formed in the gel, which causes the texture of the product to become harder or stronger until it reaches its optimum (Lestario et al., 2016; Maharani et al., 2022). If $CaCl_2$ is still added, it will destroy the gel; the newly added $CaCl_2$ will pull the $CaCl_2$ in the eggbox, destroying the crosslinking bond. Therefore, the texture of the samples treated with 0.5% CaCl2 decreased sharply because of the destruction of the crosslinking and egg box structures.

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Anthocyanin Content

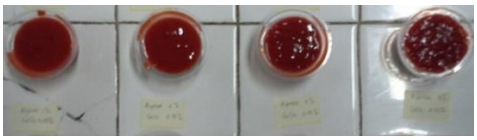
The anthocyanin content of the restructurized strawberry fruit is presented in Table 1. The results showed that the higher the concentration of $CaCl_2$, the lower was the anthocyanin content. However, the differences between the concentrations were not significant. Increasing the concentration of $CaCl_2$ tended to decrease the anthocyanin content of restructurized strawberry fruit slightly, but not significantly.

Furthermore, the visual appearance did not show a decrease in the purple/red color, which reflected a decrease in anthocyanin. with increasing $CaCl_2$. It was estimated that the anthocyanin was still in the product or not degraded with the increasing CaCl2 concentration. However, it was not extracted in anthocyanin determination because anthocyanin compounds can be bound to calcium alginate hydrogels (eggbox alginate-$CaCl_2$) through ionic interactions, where calcium ions (Ca^{2+}) help form a strong alginate gel structure and also interact with anthocyanins, causing some anthocyanins to be trapped or bound within the gel matrix, resulting in reduced levels when measured using standard extraction methods of anthocyanins. However, the non-extracted anthocyanin was still in the product, and when the restructurized fruit was consumed, the anthocyanin was still in the product and can benefit the human body as an antioxidant (Herawati et al., 2016).

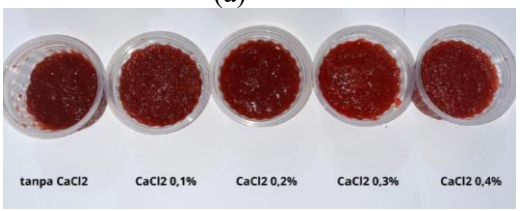
Water Content

The higher concentration of $CaCl_2$, affected insignificantly on water content of the product Table 1.). It decreased in 0.1% $CaCl_2$ but increased in 0.2% $CaCl_2$ and then decreased again in 0.3% $CaCl_2$. In other words, the water content significantly increased with increasing $CaCl_2$ content, from 76.76 to 80.12%.

According to the Indonesian National Standard (SNI), the water content of 'jelly like' food should be around 20%. The water content in this study was too high; therefore, it needs to be developed again to reach the Indonesian National Standard. However, the appearance of the product was satisfactory to the panelists in the organoleptic test. They did not appear too moist and looked better and former compared (than) those in previous studies (Figure 1a and 1b).



(a)



(b)

Figure 1. Appearance of restructurized strawberry fruit. Previous researh (a), this research (b)

Organoleptic Test

The results of the organoleptic tests of the restructurized strawberry fruit are presented in Table 2. The results of the organoleptic test showed that the higher the concentration of $CaCl_2$, the color preference of the panelists was not affected; however, it did have an effect on the aroma, texture, taste, and overall. The aroma had tendency to decrease or less prefered by the panelist, the most prefered for aroma was 0.1% $CaCl_2$; The most prefered texture was 0.2-0.4% $CaCl_2$; the most prefered taste was 0.1-0.3% $CaCl_2$; the most prefered for overall was 0.4% $CaCl_2$. From all the results of the organoleptic test, the most important factor was taste; therefore, 0.4% $CaCl_2$ could be the best concentration.

Shelf Life

The results of the shelf life test of restructurized fruit can be seen in Table 3, which showed that the product can be stored in the refrigerator for up to 9 days. From day 0 to day 9, the color was red in all concentrations, there was no mold, the aroma was the same as at the beginning (strawberry aroma), and the flavor/taste was sweet and slightly sour.

However, on day 12th, the color of the products was still red, but some part of the surface of the product with 0.0, 0.1, 0.2, and 0.3% $CaCl_2$ was covered by white mold, whereas the product with 0.4% and 0.5% $CaCl_2$ was not covered by mold. This can be explained by the fact that the products with

0.4 and 0.5% $CaCl_2$ had higher Aw than those with 0.0, 0.1, 0.2, and 0.3% $CaCl_2$. This is in accordance with the theory that the higher the Aw, the more difficult it is for microbes to grow (Winarno, 1987). The aroma became sour at all concentrations, indicating that the products began to decay; the flavor was not tested for safety. In future research, shelf life observations will be performed every day to obtain a higher thorough. In this case, the product may have still been good on days 10 or 11.

Table 2. Effect of calcium chloride concentration on organoleptic characteristics of the restructurized strawberry fruit

$CaCl_2$ (%)	Color	Aroma	Texture	Taste	Overall
0.0	3.60±0.67^{a}	3.70±0.84^{b}	2.53±0.63^{b}	3.70±0.95^{b}	3.50±0.51^{b}
0.1	3.67±0.66^{a}	3.70±0.84^{b}	2.87±0.51^{bc}	3.57±1.01^{b}	3.50±0.51^{b}
0.2	3.73±0.69^{a}	3.60±0.81^{ab}	3.07±0.37^{c}	3.60±0.62^{b}	3.77±0.77^{b}
0.3	3.73±0.64^{a}	3.57±0.86^{ab}	3.03±0.93^{c}	3.60±0.86^{b}	3.57±0.50^{b}
0.4	3.53±0.73^{a}	3.37±0.89^{ab}	3.17±0.59^{c}	3.50±0.78^{b}	4.20±0.76^{c}
0.5	3.37±0.81^{a}	3.13±1.04^{a}	2.03±0.76^{a}	2.50±0.78^{a}	2.63±0.56^{a}

Notes: Data (mean±SD) were interval data from transformed ordinal data of organoleptic hedonic responses. The data were analyzed using ANOVA. The data within the same column followed by different letters are significantly different (LSD test, $p<0.05$).

Table 3. Shelf life test of restructurized fruit

Day	Color	Microbes	Aroma	Flavor
0	Red	-	Fruity (Strawbery)	Sweet
3	Red	-	Fruity (Strawbery)	Sweet
6	Red	-	Fruity (Strawbery)	Sweet
9	Red	-	Fruity (Strawbery)	Sweet
12	Red	0%-0.3%: + 0.4%-0.5%: -	Sour +	Not tested
15	Red, covered by slighty white microbes	0%-0.3%: ++ 0.4%-0.5%: +	very sour ++	Not tested

Strawberries are fruits with a very short shelf life, often classified as a 'very perishable' fruit with characteristics of high water content, soft and thin peel, and high ethylene production before ripening, which results in a high respiration rate, and therefore easy decay (perishable). Therefore, this type of fruit requires good handling to extend its shelf life or to make a product based on this fruit with no heat to avoid the decrease in nutrition, including anthocyanin and vitamin C. This method is called restructurization, and the product is called restructurized fruit, which extends the shelf life with minimal processing. Compared to the original fruit, which usually only lasts 1-2 days in the refrigerator, the restructurized strawberry fruit can extend the shelf life up to 9 days in the refrigerator.

On the 12th day, the appearance became moldy on the surface area, covered by slightly mold; the aroma became sour; and the taste was not tested due to safety. Therefore, it can be concluded that the shelf life of the restructurized product was nine days. However, the product was still good on day 10 or 11, but not observed. Further research will observe the product every 2 days or every day to obtain more accurate data.

CONCLUSIONS

The optimal concentration of $CaCl_2$ that produced the best restructurized strawberries was 0.4% (for 2% sodium alginate). At this concentration, the anthocyanin content was 22.81 ± 4.69 mg/100 g; the texture score was 3.17 ± 0.76 (scale 1-5, hardest among other concentrations); for sensory acceptability, the

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color score was 3.53±0.73; aroma was 3.37±0.89; texture was 3.17±0.59 (most preferable compared to other concentrations); taste was 3.50±0.78; overall was 4.20±0.76 (most preferable compared to other concentrations). In addition, this study yielded better results than previous studies, especially in terms of firmer texture and longer shelf life (from 1 d to 9 days).

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