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The effect of blanching and microwave pasteurization on bioactive compounds and alpha-amylase inhibitory activity of Carica fruit (*Carica pubescens*) puree

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Abstract

Blanching and pasteurization are used in fruit processing to inactivate enzymes without damaging bioactive components. This study aimed to determine the effects of blanching and microwave pasteurization on bioactive compounds (total flavonoid, β-carotene, quercetin content) and α-amylase inhibitory activity of *Carica pubescens* puree. UV-Vis Spectrophotometry measured total flavonoid, β-carotene, and α-amylase inhibitory activity, while HPLC measured quercetin content. The data were analyzed using a one-way ANOVA and Tukey HSD test. Blanching, pasteurization, and a combination of both in *C. pubescens* puree increased total flavonoid content from 16.94 mg QE/100 g to 37.42 - 82.23 mg QE/100 g and β-carotene contents from 668.22 µg/100 g to 971.51 – 1153.94 µg/100 g. On the other hand, the treatments decreased the quercetin contents and α-amylase inhibitory activity to 21.95-76.68% and 6.05-19.73%, respectively. Pasteurization was the best treatment compared to control, blanching, and the combination of blanching and pasteurization because it offered the highest bioactive components (total flavonoids and β-carotene).

1. Introduction

Fruits are rich in vitamins, minerals, and contain some bioactive compounds that can increase the body's protection against diseases (Devalaraja *et al*., 2011). *Carica pubescens* is a variety of fruit grown in Dieng, Central Java, Indonesia, locally referred to as Carica or mountain papaya. *C. pubescens* grows at an altitude of 2000 meters above sea level (masl) in conditions of low temperature and heavy rainfall (Laily *et al*., 2012). The popularity of *C. pubescens* has increased as a result of its mouthwatering flavor and texture, nutritional benefits, and therapeutic uses (Vega *et al*., 2019).

Carica pubescens contains secondary metabolite substances such as flavonoid, quercetin, glycosides rutin, β-carotene, 6'-O-malonylated a-D-glucopyranosides of benzyl alcohol, 2-phenylethanol, and geraniol (Simirgiotis *et al*., 2009). Antioxidant capacity in *C. pubescens* (IC_{50} of 0.983 mg/100 mL) was higher than in *C. papaya* (IC_{50} of 5.326 mg/100 mL) (Laily *et al*., 2012). This compound can be used to produce a functional food, such as a puree. The high demand for fruit juice in previous years was due to the increased consumer awareness about the health potential offered (Duffett, 2018). Preview studies have proven that consumers' mindsets grow better, which affects their food choices and preferences (Sharma, 2015).

Various juice processing procedures were implemented to make high-quality and safe commercial products. Blanching and pasteurization are the two critical steps in the preparation of puree (Echavarría *et al*., 2011). Blanching aims to inactivate enzymes inside the fruit, while the pasteurization process aims to extend the shelf life (McLellan and Padilla-Zakour, 2004; Xiao *et al*., 2017). Microwave pasteurization could be an alternative heat treatment to reduce the burden of pathogenic microbes without damaging the bioactive compounds (Cañumir *et al*., 2002; Beegum *et al*., 2019). According to Saikia *et al.* (2016), microwave pasteurization elevated the total flavonoid content in litchi juice from 14.50 mg QE/100 mL to 25 mg QE/100

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mL and in black jamun from 58.38 mg QE/100 mL to 81.75 mg QE/100 mL compared to the control.

However, there is no research regarding the effect of blanching and pasteurization on *C. pubescens* puree. This research is essential to determine the impact of the blanching and microwave pasteurization process on bioactive compounds, namely total flavonoid, *β*-carotene, quercetin content, and α-amylase inhibitory activity in *C. pubescens* puree. The study's findings could be used as a reference to determine the best procedure for processing *C. pubescens* puree.

2. Materials and methods

2.1 Materials

The raw material of *C. pubescens* was obtained from Batur, Dieng, Banjarnegara regency, Central Java, at an altitude of 2000–2020 masl. The selected fruits have approximately 90% maturity, as indicated by the fruit peel color, which is almost perfect yellow. The sample of *C. pubescens* puree was divided into four treatments (Figure 1).

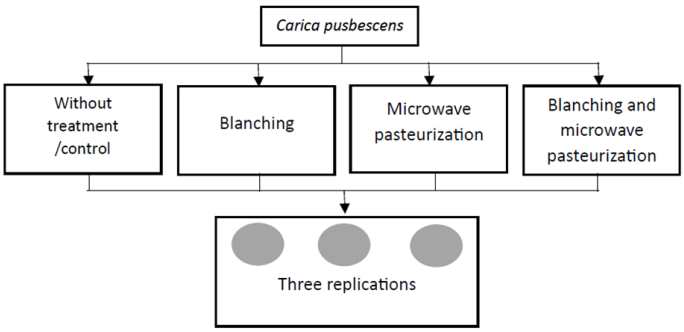


Figure 1. The treatment of *C. pubescens.*

2.2 Sample preparation

The control sample: *C. pubescens* (100 g) was peeled, and the seeds were removed. The flesh of *C. pubescens* was soaked in salt water for 60 s. Then, drained and cut into cubes. The pieces of *C. pubescens* flesh were blended until homogeneous and packed in glass bottles.

The blanching treatment: *C. pubescens* (100 g) were peeled, and the seeds were removed. The flesh of *C. pubescens* was soaked in salt water for 60 s. The flesh is drained and cut into cubes. The fruit was blanched in water at 95°C for 10 s. After the blanching process, the fruit was cooled in an ice bath until it reached 37°C. *C. pubescens* flesh was blended until homogeneous and packed in glass bottles.

The microwave pasteurization: *C. pubescens* (100 g) were peeled, and the seeds were removed. The flesh of *C. pubescens* was soaked in salt water for 60 s. The flesh was drained and cut into cubes. The pieces of *C. pubescens* flesh were blended until homogeneous and placed in glass bottles. In the final stage, *C. pubescens* puree was pasteurized using a microwave set to 900 W for 30 s. The puree was chilled in an ice bath to reach 37°C, then the bottle was sealed.

The blanching and microwave pasteurization: *C. pubescens* (100 g) were peeled, and the seeds were removed. The flesh of *C. pubescens* was soaked in salt water for 60 s. The flesh is drained and cut into cubes. The fruit was blanched in water at 95°C for 10 s. After the blanching process, the fruit was cooled in the ice bath until it reached room temperature. *C. pubescens* flesh was blended until homogeneous and placed in glass bottles. In the final stage, *C. pubescens* puree was pasteurized using a microwave set at 900 watts for 30 s. Then, remove *C. pubescens* puree from the microwave, cool inside the ice bath until 37^{o}C, and seal the bottle.

2.3 Total flavonoids content

The procedure of total flavonoids content (TFC) was conducted in triplicate by a spectrophotometric method based on the formation of aluminum-flavonoid complex (Chandra *et al*., 2014). A total of 5 g of sample was dissolved in 100 mL ethanol, then the solution was centrifuged to separate insoluble solids. One mL of supernatant was added to 3 mL of 5% $AlCl_3$ solution. The solution was diluted until the volume reached 10 mL. The absorbance was measured at a wavelength of 510 nm. Standard curves were made using quercetin at 6 different concentration points. Quercetin stock solution made by diluting 15 mg of quercetin in 100 mL of ethanol (concentration = 0.15 mg/mL). The result of the calculation is expressed as mg quercetin equivalent (mg QE/100 g) of *C. pubescens* puree.

2.4 Beta-carotene content

The sample (1 g) was dissolved in petroleum ether and acetone (1:1, v/v). Approximately 50 mL of distilled water was added and the mixture was allowed to stand for 5 min to form two layers. The upper layer consisted of the carotene fraction in petroleum ether, while the lower layer contained the remaining acetone fraction that was not dissolved in water. The lower layer was removed, and the upper layer was transferred into an Erlenmeyer flask. Sodium sulfate (Na_2SO_4) was then added. After that, petroleum ether was added until the volume reached 5 mL. The absorbance was measured using a spectrophotometer at a wavelength of 450 nm.

2.5 Quercetin

Quercetin content was determined using High-Performance Liquid Chromatography (HPLC) analysis

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(Park *et al*., 2016). *Carica pubescens* puree (25 g) samples were filtered to obtain the puree extract. Quercetin standard stock solution was made by dissolving quercetin powder in methanol to obtain a calibration standard solution with concentrations of 0, 25, 50, 100, and 200 ppm. Quercetin levels were tested by injecting 1.5 mL of *C. pubescens* puree into an HPLC vial. The chromatographic separation process (stationary phase) was achieved using a C-18 column (150 mm, 3.0 mm, 2 mm) with the column temperature maintained at 45°C. The reverse phase was performed using gradient elution with a 0.3 mL/min flow rate. Methanol with 100% concentration was used as the mobile phase. The total injection volume is 20 mL per solution, with a total running time of 10 min. The absorbance was monitored at a wavelength of 254 nm. The data were processed with the Shimadzu Lab Solution software.

2.6 The alpha-amylase enzyme inhibitory activity

The α-amylase enzyme inhibitory activity was performed using the chromogenic method adopted from Sigma–Aldrich (Kazeem *et al*., 2013). The α-amylase enzyme inhibitory activity test was carried out by dissolving 10 mg of the *C. pubescens* puree in 10 mL of ethanol to obtain a concentration of 1000 ppm. The sample mixture and the solvent are then homogenized using a vortex. Approximately 250 µL of extract (1 mg/ mL) was put into a test tube. Then, to the test tube, a 250 µL solution consisting of α-amylase enzyme and phosphate buffer (0.02 M Na_2HPO_4 and 0.067 M NaCl at pH 6.9) with a ratio of 1:2 was added. The mixture was then pre-incubated for 10 min at 25°C. A total of 250 µL of 1% starch solution on phosphate buffer (pH 6.9) was added to the test tube and then incubated for 10 min at 25°C. The reaction was stopped by adding 500 µL of dinitrosalicylic acid reagent (20 mL 96 mM 3.5-dinitrosalicylic acid, 12 g sodium potassium tartrate in 8 mL 2 M NaOH, and 12 mL distilled water). The mixture in the test tube was incubated for 5 min in boiling water (85-90°C) and then cooled to 37°C. The reaction is diluted with 5 mL of distilled water. The absorbance was read using a UV-Vis spectrophotometer at 540 nm. Acarbose 1 mg/mL, which is an anti-diabetic drug, was used as a comparison

2.8 Statistical analysis

The quantitative data obtained were analyzed using the IBM SPSS Statistics 25. The data were analyzed using One-way ANOVA, and a post hoc test was carried out using Tukey HSD. The analysis was performed with a 95% confidence level with a p-value of 0.05 and α = 0.05.

3. Results

3.1 Total flavonoids content

The total flavonoids of *C. pubescens* are shown in Table 1. The total flavonoid content in *C. pubescens* puree was significantly different (p<0.05). The total flavonoid content from the largest to the smallest was pasteurization > blanching and pasteurization > blanching > control. Pasteurization treatment had the highest amount of total flavonoids with 82.23 mg QE/100 mL fruit puree, and the lowest amount of total flavonoids were found in control puree with 16.94 mg QE/100 ml fruit puree. Blanching treatment can increase the total flavonoid in *C. pubescens* puree from 16.94 mg QE/g to 37.42 mg QE/g.

Table 1. Total flavonoid in *C. pubescens* puree.

Treatment	Flavonoid total (mg QE/100 g)	p-value
Control	16.94±1.48[a]	0.000
Blanching	37.42±3.70[b]	
Pasteurization	82.23±11.14[c]	
Blanching and pasteurization	60.10±5.61[d]	

Values are presented as mean±SD. Values with different superscripts are statistically significantly different (p<0.05) using a one-way ANOVA test.

3.2 Beta-carotene

Beta-carotene in *C. pubescens* fruit puree had a significant difference (p<0.05) (Table 2). The treatment of blanching, microwave pasteurization, and a combination of both significantly increased the β-carotene of *C. pubescens* fruit puree. *Carica pubescens* puree with pasteurization had the highest amount of β-carotene with a value of 1153.94 µg/100 g, followed by blanching that had β-carotene with a value of 1008.02 µg/100 g. While the combination of blanching and pasteurization treatment had β-carotene with a value of 971.51 µg/100 g and the lowest β-carotene was in the control treatment, with a value of 668.22 µg/100 g.

Table 2. Beta-carotene in *C. pubescens* puree.

Treatment	Beta-carotene (µg/100 g)	p-value
Control	668.22±29.70[b]	0.000
Blanching	1008.02±105.93[a]	
Pasteurization	1153.94±84.90[a]	
Blanching and pasteurization	971.51±68.48[a]	

Values are presented as mean±SD. Values with different superscripts are statistically significantly different (p<0.05) using a one-way ANOVA test.

3.3 Quercetin

Based on the results in Table 3, there was a significant difference in the quercetin content in *C. pubescens* puree (p<0.05) in each treatment. The highest

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quercetin content was found in the control puree with a value of 49.06 mg/L, while the lowest levels of quercetin were in a combination of blanching and microwave pasteurization puree with a value of 11.44 mg/L.

Table 3. Quercetin contents of *C. pubescens* puree.

Treatment	Quercetin contents (mg/L)	p-value
Control	49.06±15.37[a]	0.023
Blanching	17.11±8.44[ab]	
Pasteurization	38.29±16.33[ab]	
Blanching and pasteurization	11.44±9.95[b]	

Values are presented as mean±SD. Values with different superscripts are statistically significantly different ($p<0.05$) using a one-way ANOVA test.

3.4 Alpha-amylase inhibitory activity

The data in Table 4 showed a significant difference in α-amylase inhibition in *C. pubescens* puree ($p<0.05$). Control puree had the highest inhibitory activity of α-amylase, with a percentage of 94.23%, while the lowest inhibitory activity was owned by blanching puree, with a percentage of 75.64%. Blanching, microwave pasteurization, and the combination had α-amylase inhibitory activity higher than acarbose (control).

Table 4. Alpha-amylase inhibitory activity of *C. pubescens* puree.

Treatment	Alpha-amylase Inhibitory Activity (%)	p-value
Control	94.23±0.42[a]	0.000
Blanching	75.64±3.22[a]	
Pasteurization	80.49±7.14[a]	
Blanching and pasteurization	88.10±5.64[a]	
Acarbose	54.01±8.85[b]	

Values are presented as mean±SD. Values with different superscripts are statistically significantly different ($p<0.05$) using a one-way ANOVA test.

4. Discussion

Blanching and pasteurization are heat treatments commonly used to process fruit puree. They are critical steps aimed at improving the texture, regulating the natural color of the product, inhibiting enzymatic and microbial activity, removing trapped respiratory air in the material, and dissolving protopectin (Jabbar *et al*., 2014; Featherstone, 2015). Therefore, the effects of blanching and microwave pasteurization on total flavonoids, β-carotene, quercetin contents, and α-amylase inhibitory activity of *C. pubescens* puree were evaluated in this study.

The total flavonoid of *C. pubescens* puree can be increased up to 485.4% by pasteurization treatment. This result was similar to research by Saikia *et al.* (2016) that microwave pasteurization increased the total flavonoid content of litchi juice from 14.50 mg QE/100 mL up to 25 mg QE/100 mL and black jamun from 58.38 mg QE/100 mL up to 81.75 mg QE/100 mL, compared to the control. Pasteurization can maintain the nutritional content and sensory characteristics of the material due to quick heat transfer (Ahmed and Ramaswamy, 2020)

Blanching treatment can increase the total flavonoid in *C. pubescens* puree from 16.94 mg QE/g to 37.42 mg QE/g. The result of this study was similar to the research by Israel *et al.* (2020), that the amount of total flavonoid in half-ripe bignay (*Antidesma bunius*) increased from 10 mg QE/g to 12 mg QE/g after blanched at 95°C. The decrease in enzyme activity was responsible for the rise in total flavonoids caused by the destruction of nutritional content during the blanching process. The blanching process can inhibit flavonoid compounds' phenolic oxidation (browning). The polyphenol oxidase (PPO) and peroxidase (POD) enzymes led to the browning mechanism. PPO catalyzes the oxidation of polyphenols to o-quinones in the presence of oxygen. O-quinone polymerizes into a brown, red, or black pigment, which can affect the nutritional quality (including bioactive content) and the sensory properties of a product (Zhang and Shao, 2015; Minatel *et al*., 2017; Taranto *et al*., 2017). One of the main factors that affects polyphenol oxidase activity is pH, and the optimum pH of PPO is 4.0-8.0. *C. pubescens* has a pH in the range of PPO's optimum pH with a value of 4.1 (Uribe *et al*., 2015).

Beta-carotene from *C. pubescens* fruit puree with microwave pasteurization was higher than blanching or a combination of both. Microwave pasteurization can degrade food cell membranes and increase β-carotene extraction (Benlloch-Tinoco *et al*., 2015). Beta-carotene in *C. pubescens* puree increased up to 72.7% after pasteurization due to the enhanced extraction efficiency from heat treatment. High-temperature processing results in the oxidation and isomerization of β-carotene, which influences its concentration and bioavailability. The study by D'evoli *et al.* (2013) showed that pasteurization can increase β-carotene content in fresh carrots.

Blanching increased β-carotene in *C. pubescens* up to 50.8%. Blanching causes moisture loss and soluble solids, further growing the present carotenes. The inhibition of some oxidative enzymes causes the breakdown of specific structures, increasing the bioavailability of β-carotene (Dutta *et al*., 2005).

Quercetin content in *C. pubescens* puree from the smallest to largest was blanching and pasteurization < blanching < pasteurization < control. The reduction of quercetin from *C. pubescens* puree in Table 3 was due to

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the sub-optimum extraction process. The solvents play an essential role in the extraction process of phenolic compounds (Ngo *et al*., 2017). Quercetin is relatively labile to degradation due to the unsaturated double bonds, and also because of hydroxyl and ketone groups. The majority of research found that pasteurized veggies had less quercetin (Peng *et al*., 2017).

The α-amylase inhibitory ability of the blanch and microwave pasteurization puree decreased to 6.05% and 14.58%, respectively. The study of Oboh *et al.* (2012) revealed that the blanching process decreased the IC_{50} value of α-amylase inhibitory activity *Telfairia occidentalis* from 0.17 mg/mL to 0.24 mg/mL. The phenolic acids and flavonoids present in *C. pubescens* include rutin, caffeic acid, chlorogenic acid, cinnamic acid, ferulic acid, and p-coumaric acid (Simirgiotis *et al*., 2009; Laily and Khoiri, 2016; Sasongko *et al*., 2018), which also play a role in inhibiting the α-amylase enzyme (Xiong *et al*., 2020). The blanching and pasteurization process caused several nutrient losses/ damage, thereby reducing α-amylase inhibitory activity. According to a study by Saetan *et al.* (2016), showed that blanching at 100°C for 60 s reduced ferulic and cinnamic acid levels in *Cinnamomum porrectum* herbal tea by 41.32% and 58.87%.

This study provides valuable insights into the effects of blanching and microwave pasteurization on the bioactive compounds and enzyme inhibitory activity in *C. pubescens* puree. Significant benefits were highlighted, including increased levels of β-carotene and flavonoids, but there were also some disadvantages, such as decreased quercetin levels and α-amylase inhibitory activity. Although there are limitations to the findings and the need for more research to optimize processing techniques for optimal nutritional outcomes, the findings are valuable for the food processing sector. The study does not include sensory analysis to determine the impact of blanching and pasteurization on the taste, texture, and overall acceptability of the puree, which are important factors for consumer acceptance.

5. Conclusion

Blanching, pasteurization, and a combination of both in *C. pubescens* puree increased total flavonoid content from 16.94 mg QE/100 g to 37.42 - 82.23 mg QE/100 g and β-carotene contents from 668.22 µg/100g to 971.51 – 1153.94 µg/100 g. Furthermore, the treatment decreased the quercetin up to 21.95-76.68% compared to the control. Control puree in *C. pubescens* had the highest α-amylase inhibitory activity with a percentage of 94.23%. In comparison, the lowest inhibitory activity was owned by blanching puree, which had a rate of 75.64%. Pasteurization was the best treatment compared to control, blanching, and the combination of blanching and pasteurization because it had the highest bioactive components (total flavonoids and β-carotene).

Conflict of interest

The authors declare no conflict of interest.

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