Effect of Air Cooling and Vacuum Cooling Storage on the β-Carotene Content and Proximate Analysis (Water Content, pH, Total Protein and Content of Sugar) in Carrot

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Abstract. The study of air cooling and vacuum cooling storage effect on the β-carotene content and proximate analysis in carrot has been studied. The aim of the research to determine the effective storage in carrot to improve the quality and the shelf life. Parameters measured during the 12 weeks of storage process were β-carotene, pH, water, sugar and protein content. Validation analysis for β-carotene method showed a good linearity ($r^2 = 0.997$) in a range of 0-8 mg/L and ($r^2=0.999$) in a range of 0-1 mg/L. The precision was exemplified by %RSD of 0.88%-7.48%. Mean recovery was 100.66% during accuracy studied. UV analysis revealed the LOD values were 0.009 mg/L and LOQ values were 0.032 mg/L. The decreased content of β-carotene, water, protein, and pH from carrot during vacuum cooling storage were higher than in the air cooling storage period. The sugar content for air cooling storage increased up to eight weeks and decreased at the end of storage while the vacuum cooling storage decreased from the beginning of the storage period. All the data indicates that the air cooling storage was more effective storage techniques for extending the shelf life of carrot compared to the vacuum cooling storage.

1. Introduction
Deficiency of vitamin A is major cause of premature death in developing country, particularly among children. According to the WHO around 250,000-500,000 the children not growing up because lack of vitamin A. Vitamin A has many systemic functions in humans can be produced within the body from carotenoids, notably β-carotene [1]. Carrot has high β-carotene about 80% of total carotenoid contain [2] which will converted into vitamin A. The wrong post-harvest handling can reduce its quality, nutrition and the shelf life of carrot because of physical, chemical, and microbiological effects [3]. One of chemical effect is a respiration process [4], which makes improvement in decay process of carrot due to activity of microorganism increase.

Therefore, a storage technique is needed to improve the quality and the shelf life of carrot. A storage techniques has been do are air cooling and vacuum cooling storage, because low temperature can extend shelf life and maintain the nutritional quality of carrots, by inhibiting the maturation process [4-6]. Vacuum storage tends to reduce the growth of aerobic bacteria during storage period because its cannot do respiration prose due to lack of O$_2$ [7, 8]. In the vacuum cooling storage, the selection of the best packaging material
is a crucial point for extend the food shelf-life. Therefore, this research used polivinyl chloride (PVC) and nylon plastic for vacuum process because those are have a low permeability to H$_2$O so it's hard to absorb water from outside to the system [9]. Parameters measured from the both of storage that may occur during 12 weeks the storage period are β-Carotene content and proximate content include water content, pH, protein content and sugar content.

2. Experimental

2.1 Proximate Analysis

Proximate analysis for water content using oven method based on SNI-01-2891-1992, sugar content using Lufft School method based on SNI 01-2892-1992, pH test based on SNI-01-2891-1992 and protein assay using semi micro kjeldahl method based on AOAC.

2.2 β-Carotene Analysis

β-Carotene analysis was carried out using UV-Vis spectrophotometer by modified procedure from Karnjawanipagul research [10]. Briefly, 40 g of carrot was blended with 4 g anhydrous sodium carbonate and mixed with a mechanical blender. 5 g of the mixture was transferred into a centrifuge tube, added with 10 mL tetrahydrofuran and mixed for 24 hours at 5°C. The mixture was centrifuged at 1000 rpm for 30 min and the supernatant was collected. Extraction was performed by adding 7.5 mL dichloromethane and 7.5 mL of 10% w/v NaCl into the supernatant and shaken for 2 min. The extraction was repeated twice, organic layer was collected and evaporated under nitrogen steam. The residue was kept at 5°C, 0.1 mL of residue reconstituted with 5 mL DCM for UV measurements.

2.3 Method of Validation

Validation method was evaluated in terms of linearity, accuracy, precision, limits of detection (LOD) and limits of quantitation (LOQ). Linearity of the method was performed in a range of 0-8mg/L and 0-1mg/L standard β-carotene solution which diluted in dichloromethane solvent. Precision was determined from repeatability, intra-day and inter-day precision and relative standard deviation (RDS) was calculated. Repeatability was from repetitive UV measurement of standard β-carotene solution at 4 mg/L (n=6). Intra- and inter-day precision was determined from UV measurement of standard β-carotene solution at 4 mg/L on the same day (n=10) and on different days (n=6). Accuracy was performed by standard addition method and recovery (R) was calculated. %R was calculated from (amount found/amount added) x 100. LOD and LOQ were calculated from (3 x SD)/s and (10 x SD)/s, where SD and s were standard deviation of blank measurement (n=7) and slope of calibration curve, respectively.

3. Result and Discussion

3.1 Analysis Proximate

3.1.1. Physical Changes during the Storage Period

During the both of storage physical changes that occur were a decrease the intensity of color and texture of carrot (Table 1). In the air cooling storage a black rot was occur at the surface skin of carrot in week 12, while in the vacuum cooling storage has occurred in week 4 of the storage period. In the air cooling storage a black rot was occur at the surface skin of carrot in week 12, while in the vacuum cooling storage has occurred in week 4 of the storage period.

3.1.2. Water Content of Carrot during Storage Process

The water content in the air cooling storage decreased by 2.67% over 12 weeks of storage period (Figure 1) due to microbial unable to do respiration process and transpiration process especially water at fiber tissue, because this type of water is easy to evaporated [11]. This storage inhibited activity of pathogenic bacteria L.monocytogenes, Salmonella enteritidis, and C.sakazakii [12]. While in the vacuum cooling storage decreased by 0.6% over the four weeks of storage and had increased 0.57% for twelve weeks of storage. That showed vacuum process only inhibited aerobic respiration for 4 weeks of vacuum cooling storage and had been working out as usual caused by the activity of Pseudomonas sp, because these bacteria keep running its activities in low temperatures [13].
Table 1. Physical changes of carrot during the storage period.

|             | 0 weeks | 4 weeks | 8 weeks | 12 weeks |
|-------------|---------|---------|---------|----------|
| Air Cooling | ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) | ![Image](image4.png) |
| Storage     |         |         |         |          |
| Vacuum      | ![Image](image5.png) | ![Image](image6.png) | ![Image](image7.png) | ![Image](image8.png) |
| cooling     |         |         |         |          |
| Storage     |         |         |         |          |

Figure 1. Water content of carrot during air cooling (red) and vacuum cooling (green) storage period.

Figure 2. pH value of carrot during air cooling (red) and vacuum cooling (green) storage period.

3.1.3. pH value of Carrot during Storage Process

pH value of carrot during storage process showed at figure 2. The decrease of pH during the both of storage is estimated because of anaerobic respiration process by bacteria *L. mesenteroides*, these bacteria can do anaerobic respiration at a temperature of 2 °C [14]. The decreased of pH value from carrot on 12 weeks storage period vacuum cooling were higher than the carrot in the air cooling storage, on the air cooling storage was 6.4 to 4.9 and on the vacuum cooling storage was 6.4 to 3.5. That happened because vacuum condition by lowering the concentration of O2 will increase the growth of fungus [15] in carrots and cause anaerobic respiration which produces excess CO2 and lactic acid that caused decrease in the pH value.

3.1.4. Protein Content of Carrot during Storage Process

Protein content of carrot during air cooling storage decreased by 75.39% while in the vacuum cooling storage decreased by 95.17% (Figure 3). Protein content during the both of storage decreased caused by
coagulation and denaturation process due to changes in pH and cold temperatures. Denaturation occur lower temperature and lower pH will improve the process of protein denaturation [16].

Figure 3. Protein content of carrot during air cooling (red) and vacuum cooling (green) storage period.

Figure 4. Sugar Content (circle maltose, triangle lactose, rectangular glucose and fructose) of Carrot during air cooling (red) and vacuum cooling (green) storage period.

3.1.5. Sugar Content of Carrot during Storage Process
Sugar content of carrot during storage process showed at figure 4. The sugar content increased up to 8 weeks at the air cooling storage because of starch hydrolysis process into constituent compounds enzymatically [17] and decreased at the end of storage because the starch content is already used and enzyme activity has decreased simultaneously with the sugar content [18]. While at vacuum cooling storage decreased from the beginning of the storage period because maturation process not occur in this storage and the starch contained in carrot be used to anaerobic respiration.

3.2 Identification of Maximum Wavelength
UV spectrum of β-carotene was scanned from 350 to 800 nm and maximum absorption was obtained at 461 nm (figure 5). This was in good agreement with that reported in literatures [10]. UV spectrum of carrot extract and the standard showed the same pattern UV absorption at the same wavelength (Figure 6). This result confirmed that the extraction procedure was valid and the extract contained β-carotene.

Figure 5. UV-Vis spectra of 1-8 mg/L standard β-carotene in dichloromethane.

Figure 6. UV-Vis spectra of standard β-carotene (green) and β-carotene from carrot extract (red).
3.3 Method Validation

Linearity of the method was performed in a higher range of 0-8 mg/L showed good linearity with a regression of $y = 0.147x - 0.031$ ($r^2 = 0.997$) (Figure 7), where $x$ and $y$ were β-carotene concentration and UV absorption at 461 nm, respectively. At the lower range, the method also provided acceptable linearity with regression of $y = 0.222x - 0.002$ ($r^2 = 0.999$) (Figure 8).

Method repeatability showed RSD of 0.880%. Intra- and inter-day precision revealed RSD of 7.477% and 3.564%, respectively. RSD values of Intra-day were slightly high since β-carotene was unstable and easily degraded at room temperature. However, the other one were in acceptable ranges [19]. Method accuracy showed %Recovery was 100.66%, that showed the procedure is valid because it is not disturbed by the sample matrix and has an acceptable range [19]. LOD and LOQ were 0.009 mg/L and 0.032 mg/L, respectively. That indicates that the instrument cannot measure concentrations below 0.032 mg/L. Validation data indicated that the proposed method showed good linearity, precision, accuracy and sensitivity, which could be used for determination of β-carotene in carrot.

3.4 β-Carotene Content of Carrot during Storage Process

A decrease of β-carotene content during the both of storage presented at figure 7.

![Figure 7. β-carotene content of carrot during air cooling (red) and vacuum cooling (green) storage.](image)

From figure 7, the decrease of β-carotene content during the both of storage occurs due to the oxidation process that caused by the double bonds which contained in it is structure, and makes β-carotene is sensitive to oxidation [20,21], and a decreased of β-carotene content from carrot at vacuum cooling storage were higher (67.57%) than the carrot in the air cooling storage (56.42%) influenced by protein content and pH value. Low pH will be increasing a carotenoid oxidation by isomerization from trans into the cis [22] and protein molecules made a bonding with carotene molecules as a carotene bodies [2].

4. Conclusion

Air cooling storage was more effective storage techniques for extending the shelf life of carrot compared to the vacuum cooling storage which indicated from proximate content and β-carotene content. The decreased content of β-carotene, protein, and pH from carrot during vacuum cooling were higher than in the air cooling storage period, on the air cooling storage were 56.42%; 75.39%; 6.4-4.9 respectively and on the vacuum cooling storage were 67.57%; 95.17%; 6.4-3.5 respectively. The water content in the air cooling storage decreased by 2.67% while in the vacuum cooling storage decreased by 0.6% over the four weeks of storage and had increased 0.57% for twelve weeks of storage. The sugar content for air cooling storage increased
up to eight weeks and decreased at the end of storage while the vacuum cooling storage increased from the beginning of the storage period.

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