Pertanika J. Sci. & Technol. 27 (1): 387 - 396 (2019)

SCIENCE & TECHNOLOGY

Journal homepage: http://www.pertanika.upm.edu.my/

Effects of Various Drying Methods on the Vitamin C level of Papaya Locally Grown in Brunei Darussalam

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ABSTRACT

Papaya (*Carica. papaya* L.) belongs to the *Caricaceae* family. They are grown in almost all tropical and subtropical regions in the world. In Brunei Darussalam, papaya is a non-seasonal fruit that is locally grown. It is also common to dry papaya. Drying is the process of removing water or moisture from a product with the benefit of improving shelf-life by impeding food spoilage by microorganisms. In this research, freshly ripened papaya was subjected to various drying methods and its effect on the vitamin C levels was investigated. The drying methods include sun drying (SD), oven drying (OD), freeze-drying (FD) and deep freezing (DF). Fresh samples were also investigated and acted as the control. The determination of vitamin C levels was carried out using an accredited Association of Official Agricultural Chemists (AOAC) indophenol titrimetric method. The result showed that FD samples had the highest vitamin C levels (5.84 ± 0.83 mg/100g) while SD has the least value of vitamin C (2.96 ± 0.47 mg/100g). In conclusion, the FD method resulted in highest vitamin C levels. Therefore, the FD method serves as the best industrial application with good vitamin C retention in papaya.

Keywords: Deep freezing, freeze-drying, oven drying, papaya, sun drying, vitamin C

INTRODUCTION

Papaya is a non-seasonal fruit in Brunei Darussalam. Papaya or its botanical name *Carica papaya* Linn is commonly called pawpaw and belongs to the *Caricaceae* family (Yogiraj et al., 2014). Papaya is usually about 15 - 50 cm long, 10 - 20 cm in diameter, and weighing up to 9 kg (Ojike

ARTICLE INFO

Article history:
Received: 1 March 2018
Accepted:12 September 2018
Published: 24 January 2019

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ISSN: 0128-7680
e-ISSN: 2231-8526

et al., 2011). It is rich in orange pulp and often has orange-red, yellow-green and yellow-orange hues (Aravind et al., 2013). It has numerous seeds, small, black and covered with gelatinous aril. Papaya trees yield fruits within 5 months and can live up to 4-5 years (Orwa et al., 2009).

Drying is among the oldest methods of food preservation (Lau & Taip, 2011). The function of drying is to remove water from solids such as fruit and vegetables by evaporation or sublimation (Kurozawa et al., 2014). Drying can discharge approximately 80 - 90% of water from the fresh product and preserve large amounts of the nutrients (Kaleem et al., 2016). Drying causes reduction in water activity to lower the moisture content of foods. It also prevents the growth of microorganisms and hence reduces the rate of chemical reaction that causes spoilage (Lau & Taip, 2011). In food industries, advances in drying methods and dehydration techniques enable the preparation of a wide range of dried products and foods from fruits and vegetables.

Vitamin C is mostly found in natural products such as fruits and vegetables. Vitamin C is also known as ascorbic acid (AA) (Fatariah et al., 2015; Offor et al., 2015). Citrus fruits such as papaya are good sources of vitamin C. The edible portion of one papaya contains about 60 to 84 mg/100 g of vitamin C (Wall, 2006). In the human body, vitamin C acts as an electron donor and to stabilise tissues. It can also be oxidised to the more unstable dehydroascorbic acid and easily converted back to vitamin C through several enzyme systems such as reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent systems. It also functions as an enzyme cofactor that maintains the iron ion in the reduced ferrous (Fe^{2+}) state required for enzyme activity (Schlueter, & Johnston, 2016). However, vitamin C can be degraded depending on variables such as temperature, pH, light, storage, exposure to oxygen, contact with minerals (iron and copper), and alkali. Thus, if vitamin C is well maintained during the food drying process, other nutrients are probably also preserved (Marques et al, 2006).

The objective of this study is to compare the impact of various drying processes which includes sun drying (SD), oven drying (OD), freeze-drying (FD) and deep freezing (DF) on the levels of vitamin C. The results offer insights into effective approaches to papaya fruit nutritive value preservation which could benefit the food manufacturing industry.

MATERIAL AND METHODS

Papaya was washed, peeled and sliced longitudinally into four pieces (5± 1 mm thickness) with a stainless-steel knife. The seeds were removed. A home blender was used sparingly to cut the papaya into small pieces, and a cotton cloth was used to squeeze the juice. Papaya juices were weighed, and approximately 300 g were subjected to the different drying methods (SD, OD, DF and FD). Fresh papaya fruit was also analysed and served as the control. All of the extraction was carried out in triplicate. Several precautions were taken in order to perform the operations such as minimal light due to the instability of vitamin C. Firstly, 10 grams of fresh papaya was transferred into a 50-ml falcon tube, and its vitamin C was analysed on the same day of preparation.

For the SD method, approximately 40 grams of fresh papaya juice was weighed and spread over a metal tray, then placed on the concrete floor under the sun. Samples were subjected to SD from 08:00 to 16:00 hours (at least 8 hours). A minimum temperature of 30 - 35 ºC is required with humidity below 60% (Ahmed et al., 2013). For the FD method, freeze dryer (LABCONCO) was set to dry papaya juices with total pressure and the temperature inside the vacuum chamber of -0.102 mbar and -53 °C, respectively. The FD process was carried out by placing the extracted papaya juices into a 50ml falcon tube, which were prefrozen in a -70 °C freezer overnight. The prefrozen samples were then placed inside the FD until pure crystalline ice formed and turned into a powder-like product (Ahmed et al., 2013). For FD, 10g of the extracted papaya juices was placed in each falcon tube and processed for 3 days.

For the OD method, fresh papaya juices were spread evenly on aluminium foil and placed in a conventional laboratory oven at a constant temperature of 60 ºC. The drying time lasted for 24 hours to reach 10% moisture content (Workneh et al, 2012). The initial weight of the aluminium foil with papaya was noted before drying. For the DF method, 10 grams of papaya juice was added to a 50ml falcon tube and stored in a deep freezer at -70 and 80 °C for 24 hours (Alhamdan et al, 2018; Favell, 1998).

Reagents used in the study include L-Ascorbic acid (reagent grade, Sigma Aldrich), 2, 6-Dichloroindophenol (DCIP) sodium salt hydrate (BioReagent, Sigma Aldrich) and Trichloroacetic acid (CCl_3COOH) (Merck). L-Ascorbic acid (AA) is a white, odourless crystalline acidic substance with a molecular weight of 176.06 and is very soluble in water. Trichloroacetic acid is a colourless crystalline solid. Both act as a reagent in extraction and titration method. 2, 6-Dichloroindophenol (DCIP) is a dye used for analysis of vitamin C in this project (Sigma Aldrich).

For the analysis of vitamin C, a stock solution of 10% of Trichloroacetic acid (TCA) was prepared by dissolving 16.8g in a 1000ml bottle. A volume of 10ml of 10% TCA was then added to the tube containing the juice. The tubes must remain covered with aluminium foil. The solution was mixed by placing the tube on a shaker for 10 minutes. Then, the solution was homogenised using centrifugation at 4700 rpm (494 x *g*) for 10 minutes at 4 °C. Lastly, the supernatant was transferred to a clean 15ml Falcon tube. The supernatant was used to determine the vitamin level using the indophenol titration method. The titrimetric method used was employed in AOAC Officials methods 967.21 (Kim, 2011; Vasanth Kumar et al, 2013). Titrimetric method is used due to its simplicity to determine vitamin C in fruit juices. In addition, the reaction of indophenol dye with ascorbic acid is very fast. Since papaya have orangish colour, the end-point of the titration can be easily detected when an excess of the unreduced dye gives a rose pink color in an acid solution (Fatin Najwa & Azrina, 2017).

Recovery assay is used to confirm the accuracy of the method used. The protocol of this assay was adopted from Thermo Fisher Scientific (Thermo Scientific, 2007). In this assay, the samples were tested in three different batches. The first batch was the unspiked samples. Samples in the second batch were spiked with an ascorbic acid solution with a concentration of 6 mg/100g, and the samples in the third batch were spiked with 10 mg/100g of ascorbic acid. Each was done in triplicate.

Spiking of the sample was done by adding 1ml of ascorbic acid solution into 10g of the sample (extracted fruit juice) and then mixed. The spiked samples were then tested for vitamin C following the procedure described above. Additionally, a diluent made up of 10% TCA was spiked with a specified concentration of ascorbic acid in the second and third batches. It was further tested for vitamin C along with the other samples. The recovery percentage was calculated using the formula below:

$$\%\,Recovery = \frac{observed\ value\left(\frac{mg}{100g}\right)}{expected\ value\left(\frac{mg}{100g}\right)}\ x\ 100$$

Where,

Observed = spiked sample value

Expected = Amount spiked into sample (calculated based on assigned concentration of spiking stock and volume spiked into sample).

The results were analysed by an analysis of variance (ANOVA). One-way ANOVA was used to determine significance difference of mean value of vitamin C content ($p < 0.05$) among the samples for each method. In addition, error bars based on standard deviation is used to determine the variation of the data and not the error of the measurement (Najwa & Azrina, 2017).

RESULTS

The vitamin C content of the papaya juices was recorded in mg/ 100g of fresh weight. Figure 1 shows the bar graph presentation of the results. The result shows that a fresh sample had 5.84 mg/100g of vitamin C level. The vitamin C level in the FD samples was 8.80 mg/100g, which was significantly higher than the other drying methods, including the fresh samples. The vitamin C level in SD was 2.96 mg/100g, which was significantly lower than OD (3.44 mg/ 100g). The vitamin C levels of DF were found to be the second highest (4.56 mg/100g). These results simply mean that there was an impact on the level of vitamin C as a result of the drying. However, in this study, the vitamin C level found in papaya is lower than those previously described in the literature. The processes detailed in this research were repeated three times and similar trends were observed. In conclusion, the levels of vitamin C were significantly different among the drying methods ($P < 0.05$) and the standard deviation error bars do not overlap.

The recovery assay measured the accuracy of the spiked sample (known amount) by comparison to the sample matrix and standard diluent techniques. The mean recovery value for the spiked sample of 6 mg/100g AA with TCA extraction method was 95 ± 0.7% (Table 1). On the other hand, the mean recovery for the spiked sample of 10 mg/100g AA was 99 ± 1.2%. In summary, the TCA extraction method gave a satisfactory quantitative analysis of AA in papaya and across the range of tested concentration. It can be concluded that drying is a valid method to determine vitamin C levels.

Table 1
Recovery Assay of *Papaya Vitamin C Levels*

Spike level (mg/100g)	Expected (mg/100g)	Observed (mg/100g)	Recovery %
Unspiked	0.0	5.84	N.A
Low (6.0 mg/100g)	6.0	5.7	95
High (10.0 mg/100g)	10.0	9.9	99

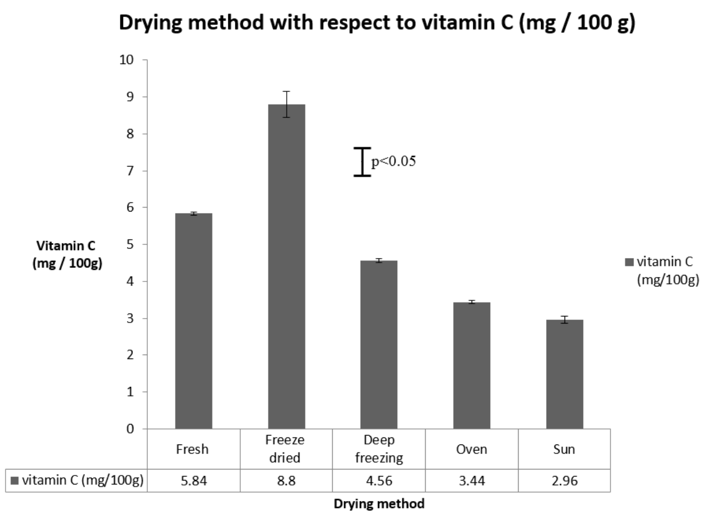

Figure 1. Level of vitamin C in mg/100g of fresh papaya and papaya that has been subjected to the various drying methods.

DISCUSSION

Several factors explain the differences in vitamin C level. It could be due to the differences in maturity stage and regional varieties of fruits (Wall, 2006). For example, male or female papaya can produce different levels of vitamin C. Differences in vitamin C levels could also be due to differences in the growing condition of the fruit (Rigi et al., 2014). For example, temperature and amount of fertiliser used in growing the plant and climatic conditions such as light can affect the vitamin C level (Tadese et al., 2014). In addition, different techniques of measuring, blending, and drying could affect the vitamin C levels of papaya (Garcia et al., 2014).

The study shows fresh papaya sample had lower vitamin C levels compared to FD papaya which could be due to the presence of moisture. Fresh papaya fruit contains about 87.67% moisture. According to the literature, the moisture content decreases with drying time (Garcia et al., 2014). Therefore, in a fresh sample, the moisture is still present, and as a result, other substance including AA is present and cause less reduction of indophenol dye. In fact, AA is the only substance in the biological material which reduces the dye (Mapson, 1942).

The FD method is recommended for drying of materials containing heat-sensitive antioxidant components such as ascorbic acid. This is because it has been found that FD products have similar characteristics to fresh products (Shofian et al., 2011). Moreover, the FD sample can preserve the nutritive value of processed products. Also, the advantage of the FD method is that it only requires low-temperature control to undergo the process (Marques et al., 2006). However, the disadvantage is its operational costs as it consumes a lot of energy and incurs costs for maintenance (Nireesha et al., 2013). Despite freeze drying retained the highest vitamin C level, freeze drying process is still expensive and limits the wide-scale application in the food industry (Ciurzyńska & Lenart, 2011).

Several factors explain why the FD sample has higher vitamin C retention compared to the other methods. Previous studies have found that overnight drying preserves a larger quantity of vitamin C than day drying (Garcia et al., 2014). Secondly, high vitamin C levels were found in the dehydrated samples due to the loss of water during drying. As a result, the high vitamin C content found in the papaya slices after drying was attributed to both the evaporation of water and the reduced loss of vitamin C during the drying process (Garcia et al., 2014).

The DF method has the second highest level of vitamin C compared to the others. This shows that vitamin C is less sensitive at refrigerated storage condition (Tirkey et al., 2014). Papaya juice stored in very cold temperatures does not lose much vitamin C compared to when stored at higher temperatures. This is because vitamin C is more sensitive to hot temperature and can easily oxidise (Tadese et al., 2014). The function of freezing is to decrease the water activity and reduce enzymatic activity resulting in extending the shelf-life of the product. During the freezing process, ice crystal is formed and cause enzymatic oxidation. The oxidation occurs due to the destruction of the cells and tissues of the product and therefore increase the contact between phenolic, oxygen and enzymes (Alhamdan et al., 2018). It can be inferred from the results of DF and FD sample that, the lower the temperature, the higher the availability of vitamin C in fruit juice. It is hence better to maintain or store vitamin C in a place below the room temperature.

For the SD method, the level of vitamin C was remarkably low compared to the other method. Papaya juices were sun-dried under varying temperatures of 24 °C – 30 °C in a day. Papaya juice was spread on a metal steel tray covered with aluminium foil and treated in open air. This could explain the low levels of vitamin C which is prone to oxidative destruction in the presence of heat, light and oxygen (Yusof et al., 2015). In addition, high temperature or exposure time of the samples during sunlight drying results in greater loss of vitamin C (Lau & Taip, 2011). This result suggests that the effect of exposure time to the air during drying was more damaging than the drying temperature itself. Thus, the low value of SD samples indicates that the vitamin C in papaya is highly sensitive to sunlight and drying.

The disadvantage of the SD method is that it is significantly slower if the air is humid. According to the finding of Santos and Silva (2008), SD is a common drying method for fruits, leaves and vegetables all over the world. One reason is that of cost. However, SD may result in poor nutrient preservation, especially for fruits having high concentrations of vitamin C (Yusof et al., 2015).

A recovery study has been conducted to confirm the validity of the method used. A standard solution of low spiked and high spiked was added to three different pre-analysed sample solutions, and the recovery of the compound was calculated. The recovery assays show that vitamin C of papaya spiked with 6 mg and 10 mg of 0.1% ascorbic acid and distilled water was successfully recovered at 95% and 99% respectively.

CONCLUSION

In this study, papaya juices were analysed for the effects of various drying methods on vitamin C levels of papaya via the AOAC indophenol method. The result showed that drying processes did have an impact on the vitamin C retention. The study also found that the most favourable drying method was FD which preserves the highest level of vitamin C. Therefore, among the various methods of drying, FD was preferred because it could be used to preserve fruits, was lightweight and increased the shelf-life of the product. The SD method is the least favourable approach as it retains the lowest amount of vitamin C.

There are some limitations to this study. The weather conditions, i.e. rain can affect the SD method. Due to rain, the papaya sample had to be discarded and the result deemed invalid causing the experiment to be repeated. Also, a portable thermohygrometer may be useful to monitor the temperature and humidity changes. Next, according to plan, the papaya sample only needed to be squeezed using a cotton cloth. However, due to difficulty during the FD procedures, the papaya had to be blend to prevent it from producing a pungent smell during the FD processes. Lastly, to obtain more specific vitamin C measurement, high-performance liquid chromatography (HPLC) could be used to eliminate the non-polar compound such as the sugars, salt, amino acid and others. From this research, further study would be to compare the vitamin C level using titration and high performance liquid chromatography (HPLC) method.

ACKNOWLEDGEMENT

We would like to thank Universiti Brunei Darussalam for providing infrastructural and academic support to run the project.

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