East African Scholars Journal of Agriculture and Life Sciences
Abbreviated Key Title: East African Scholars J Agri Life Sci
ISSN 2617-4472 (Print) | ISSN 2617-7277 (Online)
Published By East African Scholars Publisher, Kenya
Volume-8 | Issue-11 | Dec-2025 | DOI: https://doi.org/10.36349/easjals.2025.v08i11.006

OPEN ACCESS

Original Research Article

The Effects of Different Heat Preservation Methods on the Antioxidant Activity of Ambarella (Spondias Dulcis) Fruit Juice

Hanis Nadia Y[1]*, I. N. Mohd Nasir[1], L Karim[1], Hafiza Y[1]
[1]Faculty of Science and Technology, Islamic Science University Malaysia, Malaysia

Article History
Received: 28.10.2025
Accepted: 23.12.2025
Published: 26.12.2025

Journal homepage:
http://www.easpublisher.com

Quick Response Code

Abstract: Ambarella (*Spondias dulcis*) is a tropical plant with excellent phytochemicals properties and has long been used in many traditional medical practices. This study aimed to evaluate the effects of different heat preservation methods; pasteurization (85ºC in 30 seconds), water bath canning (100ºC in 10 minutes) and jar processing (121ºC in 1 minute), on the antioxidant activity of Ambarella (*Spondias dulcis*) fruit juice. The Total Phenolic Content (TPC) of Ambarella juice was determined using Folin-Ciocalteu's assay, while DPPH (1,1-diphenyl-2-picrylhydrazy) was used to analyze the free radical scavenging activity. The reducing power of Ambarella juice was assessed using Ferric Reducing Antioxidant Power (FRAP). The result of Folin-Ciocalteu's assay showed that the highest TPC value was detected in the raw juice (5.3002 ± 0.114 mg GAE/g) while the lowest was in the water bath juice (3.6137 ± 0.174 mg GAE/g). The result of free radical scavenging activity of the juice samples found that pasteurized juice exhibited the highest percentage of scavenging activity (55.81 ± 0.55%), while retorted juice was the lowest (33.44 ± 0.61%). The highest reducing power was obtained in the raw juice (98.30 ± 0.85%), while water bath juice recorded the lowest percentage of reducing power (65.66 ± 0.68%) out of all juice samples tested. The correlation between the total phenolic contents and antioxidant activities was determined as a positive, linear correlation with DPPH (r = 0.407) and FRAP (r 0.963). Similarly, a positive correlation was also observed between both of the DPPH and FRAP antioxidant assays (r = 0.625). The pasteurization method was determined as the best heat preservation method compared to water bath canning and jar processing, since it exhibited comparatively high TPC value and higher antioxidant activity percentage.

Keywords: Ambarella (Spondias Dulcis) Fruit, Heat Preservation Methods, Antioxidant Activity.

INTRODUCTION

Ambarella (*Spondias dulcis*) or also known as June Plum or Golden Apple is a tropical plant indigenous to Melanesia through Polynesia and can be widely found in equatorial and tropical countries such as Malaysia, Indonesia, Sri Lanka, Vietnam and India (Jayarathna *et al*., 2020). It belongs to Spondias genus and Anacardiaceae family, which has 18 species (Sinan *et al*., 2021). The Ambarella tree produces edible fruits that is high in carbohydrates, vitamins, and polyphenols. Moreover, it also contains numerous medicinal properties and has long been used as traditional medicine to treat diarrhoea, internal ulceration, anaemia, skin inflammation and as eye infection medication (Sarker *et al*., 2012, Islam *et al*., 2013; Sinan *et al*., 2021). The fruit's flesh is juicy, crisp, and moderately acidic, with a pineapple-like flavour and scent (Jayarathna *et al*., 2020). Hence why, Spondias dulcis is exceptionally well-known for its unique flavour profile. Once the fruit is ripened, it can be used to make juices, chutneys, jam and beverages as well as eaten raw.

Malaysia ranked 5th among the top ten exporters of "kedondong" in the world, justifying the importance of this agricultural produce, that can be used as beverage, condiment for sauces, snacks and pickles and other edible food or beverage consumption. Since this fruit can grow well in Malaysian's tropical weather, it can serve as an advantage for our country to monopoly the Ambarella market and making it as a key source to alleviate the economy using this agricultural produce.

***Corresponding Author:** Hanis Nadia Y
Faculty of Science and Technology, Islamic Science University Malaysia, Malaysia

Though Ambarella based product might not yet be as popular as other fruits such as apples or oranges, this fruit holds so many potentials that needs to be prioritised and exploited.

In addition to offering a good amount of vitamin C, folic acid, potassium, and pectin, citrus fruits like Ambarella also contain a variety of active phytochemicals that can promote health (Rahman *et al*., 2016). Antioxidants are well known for having beneficial health-promoting qualities which includes boosting immunity, slowing down the ageing process, and reducing disorders related to the metabolism. Consumption of fruits high in antioxidants was inversely correlated with risks of non-communicable diseases. Fruits and fruit juices provide a variety of nutrients that are good for health, such as ascorbic acid, phenolic compounds, vitamins, and minerals. Phenolic components, in particular, provides numerous health advantages including anti-inflammatory, anti-bacterial, anti-mutagenic, and antioxidant action (Palety *et al*., 2020).

The evolution and industrialization of the world have led to invention of novel technologies in food processing industry such as thermal treatments, canning and freezing. The purpose of these techniques is to prevent any biochemical reactions from occurring and to prevent pathogens and fungi from entering the food. The approach allows for less waste while also extending the shelf life (Pereira *et al*., 2018). These preservation techniques have contributed to major improvement in the Food and Beverages (F&B) industry by increasing the marketability and the commercial value of food products, especially for perishable foods. In addition, chemical and microbiological treatments with additives, coatings, as well as other polyphenolic plant extracts have been used in the past few years, resulting in an efficient food preservation solution (Sridhar *et al*., 2021).

One of the food products in the F&B sector that has shown a lot of improvements throughout the years in terms of preservation technologies is fruit juice. Though there are a few numbers of methods available for preserving commercialized juices drink, the methods chose must be suitable with the properties of fruit used or the end product intended for sell. Usually, manufacturers would use either chemical preservatives or thermal treatments or both. Some of the most common preservatives applied are benzoic acid (benzoates) and sulphur dioxide (sulphites) while the thermal treatments that are normally utilized are pasteurization and aseptic processing; UHT and HTST (Ramesh, 2020). The usage of these thermal treatments in the preservation of fruit juices is proved to help produce products with better sensory quality and higher nutrients retention along with minimal flavour loss.

In the manufacturing of commercialized beverages, fresh juices undergo various processing steps and preservation methods in order to extend its' shelf life. Application of heat or thermal treatments is one of the emerging technologies in preservation of food matrix. However, some preservation techniques that utilize heat treatment might affect the chemical structure of natural existing compounds in the juice, thus resulting in changes to the juice's nutritional contents. Thermal processing causes unwanted metabolic reactions and nutritional changes, which in turn lowers the quality of the product (Chen *et al*., 2013; Hu *et al*., 2020). A study by (Mahdavi *et al*., 2010; Khaw *et al*., 2016) shown that commercialized juices have much lower antioxidant activity than fresh juice. The oxidation of polyphenols during heat treatments will also lead to degradation of ascorbic acid, which is a form of Vitamin C. Additionally, carotenoids (Vitamin A) are prone to oxidation in high temperature processing. The carotenoid degradation are well associated with loss of colour in the pigment (Reinard & Maigonnat, 2012). Fresh fruit juice's antioxidant potential is different from that of its commercial counterparts for a number of reasons, including fruit type, the extraction method, processing, clarifying, filtration, and pasteurization during industrial juice production (Palety *et al*., 2020).

Therefore, analytical methods are essential to determine the effects of different preservation methods on the fresh fruit juice, specifically it's antioxidant contents and activity. The data gained from this research is beneficial to discern the best preservation method to be used in the manufacturing of commercialized fruit juice.

Experimental Section/Material and Methods

Chemicals and Reagents

For the Total Phenolic content (TPC) assay, gallic acid anhydrous that was used in preparation of stock solution was provided from Merck (Darmstadt, Germany), sodium carbonate (decahydrate) was obtained from Chemiz (Shah Alam, Malaysia) and Folin-Ciocalteu's phenol reagent was from R&M (Bhopal, India). The DPPH (2-2-diphenyl-1-picrylhydrazyl) powder utilized for DPPH assay was from Sigma Aldrich (St. Louis, USA) while the ascorbic acid for the stock solution was provided by Merck (Darmstadt, Germany). In the Ferric Reducing Antioxidant Power (FRAP) assay, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and 2,4,6-Tripyridyl-S-triazine (TPTZ) reagent was obtained from Sigma Aldrich (St. Louis, USA) while ferric chloride 6- hexahydrate (FeCl3.6H20) was from Goodrich Chemical Enterprise (Singapore). Sodium acetate trihydrate was provided from Bendosen Laboratory Chemicals (Norway), glacial acetic acid, hydrochloric acid and methanol from Merck (Darmstadt, Germany). Only analytical grade (99%) chemicals and reagents were utilized in this research.

Processing of Ambarella Juice

Fresh and ripe Ambarella fruits were stored at a cold temperature (3-4º C) in the refrigerator prior to

usage to slow down the ripening process. The fruits were washed and cleaned thoroughly with salt to remove the stains on the skin. Next, the skin was peeled and the fruits were chopped into small chunks. Approximately, 640g of the fruits were then weighed and blended together with 800ml distilled water and 100g sugar in a blender (the ratio of water to ambarella fruit is 5:4). After mixing for 10 minutes until homogenized, the juice was sieved into a jug using muslin cloth and then stored under refrigeration at a temperature of 4-5º C until further processing methods.

Preservation Methods (Pasteurization)

Two hundred and fifty millilitres (250ml) glass jars and caps were autoclaved before usage. Then, the juices will be pasteurized at 85C for 30s prior to filing it into the jars. After the bottles were loaded and sealed with plastic screw caps, they were laid flat for 2–3 minutes to pasteurize the cap, cooled in a water bath, labelled, and kept refrigerated (5°C -7°C) until further use (Wurlitzer *et al*., 2019). The process was done in triplicate.

Preservation Methods (Water Bath Canning)

250ml glass jars, caps and metal rings were sterilized using autoclave machine. The jars were kept hot before filling the juice by leaving them in hot water. Processed Ambarella juice was added into the sterile jars leaving a ¼ inch headspace. The rim of the jars was cleaned thoroughly using a sanitized cloth to make sure the sterile condition was well kept. The caps then were adjusted on the mouth of the jars and metal rings were used to seal them. Next, the water bath canner was filled half full with water and preheated to 60 °C. Once the water has achieved the temperature, water bath rack was put into the canner. The jars were placed onto the rack and more boiling water was added into the canner enough to cover the top of the jars by at least 1 to 2 inches (Barrow, 2020). The processing time was set once the water came to a boil. The water bath canning process was set for 10 minutes. After 10 minutes, the jars were removed from canner and cooled at room temperature. The jars were then labelled before stored in the refrigerator at 5-7ºC. The process was performed in triplicate.

Preservation Methods (Jar Processing)

In 250ml glass jars that were already autoclaved, approximately 240ml of Ambarella juice was filled leaving a headspace of 1/4 inch. The rim of the jars was cleaned thoroughly before the caps were placed. The jars were then put into autoclave machine to undergo jar processing at 121º C in 1 minute. After the jar process was completed, the jars were taken out and cooled in room temperature. Then, the jars were labelled and kept at cold temperature (5-7º C). This process was carried out in triplicate.

Antioxidant Activity Assay (Folin-Ciocalteu's Assay)

Folin Ciocalteu assay was used to determine the total phenolic content of fresh and heat-treated Ambarella juice. The procedure was performed based on a method in journal (Mahmood *et al*., 2011). 80% methanol was prepared by diluting 80ml methanol (99%) with 20ml distilled water. To prepare the Gallic acid stock solution, 100 mg of gallic acid were dissolved in 10 ml of 80% methanol which was then diluted with distilled water to 100 ml in a 100 ml volumetric flask to obtain a concentration of 1 mg/ml (1000 g/ml). Then, 7% sodium carbonate solution was prepared by dissolving 7g of sodium carbonate with distilled water in a 100ml volumetric flask until it reached the marker. To prepare the calibration standard solutions, the primary stock solution was diluted with distilled water to get concentrations of 50, 100, 150, 250, and 500 ug/ml of gallic acid solution. The absorbance was measured at 725nm using UV-Vis spectrophotometer and compared to a reagent blank (methanol) to obtain a standard plot. In a 25 ml volumetric flask that was consisted of 9 ml of distilled water, 1 ml of juice sample was added. The mixture was combined with 1 ml of Folin-Ciocalteu phenol reagent and mixed. Next, 10 ml of 7% sodium carbonate solution was added to the mixture after 5 minutes. The solution was then diluted with distilled water to obtain a total volume of 25ml. At 765 nm, the absorbance against the prepared reagent blank was measured with UV-Vis spectrophotometer following 90 min of room temperature incubation. The test samples were analyzed in triplicate, and the calibration curve plotted was used to calculate the sample's concentration. The result was expressed in gallic acid equivalent, mg GAE/g using the following equation:

$$T = (CxV)/M$$

Where T= total phenolic content (mg GAE/g), C= concentration of gallic acid (mg/ml), V= volume of extract solution (ml) and M= mass of extract (g).

Antioxidant Activity Assay (DPPH Free Radical Scavenging Assay)

DPPH free radical scavenging assay used in this study was assessed based on the method of (Khaw *et al*., 2016) and (Rohman *et al.*, 2010) with some adjustments. Fresh methanolic DPPH stock solution of 0.1 mM was made by dissolving 10 mg of DPPH powder in 125 mL of methanol in a 250 mL volumetric flask. The mixture was well shaken until homogeneous and the flask was then wrapped with aluminium foil. The mixture was kept in dark and cold condition prior to usage. Next, ascorbic acid stock solution was prepared by dissolving 100 mg of gallic acid in 10 ml of methanol, in a 100ml volumetric flask. The solution was then diluted with distilled water to make up a volume of 100ml, obtaining a concentration of 1 mg/ml (1000g/ml). Then, standard calibration solutions of various concentrations were prepared ranging from 25ppm to 250ppm to generate the ascorbic acid standard calibration curve. For the assay, 1ml of juice sample solutions with different concentrations (10-

50 mg/ml) was added with 3ml of DPPH solution in 10ml volumetric flasks. Methanol was added until the markup line prior to incubation at room temperature for 30min in a dark and covered place. The DPPH radical's scavenging effect was measured at 517nm with a UV-Vis spectrophotometer, and the DPPH solution absence of juice sample was used as the control. The following equation was used to calculate the radical scavenging activity as a percentage:

DPPH radical scavenging activity (%) = (1 – As/Ac) x 100

Where AC =absorbance of control and AS = absorbance of sample solution.

Antioxidant Activity Assay (Ferric Reducing Antioxidant Power)

Ferric reducing antioxidant power (FRAP) assay performed in this study was based on the procedure of (Khaw *et al*., 2016) and (Rohman *et al*., 2010) with slight modifications. The FRAP reagent was composed of 20 mM FeCl3.6H2O solution, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution, and 300 mM acetate buffer (3.1 g C2H3NaO2.3H2O and 16 mL C2H4O2). 25 mL of pH 3.6 acetate buffer, 2.5 mL of TPTZ solution, and 2.5 mL of FeCl3.6H2O solution were combined to form the new working solution. Before analysis, the mixture was heated to 37°C. Then, 1ml of juice sample with different concentrations (20-100 mg/mL) and 2 mL of reagent were put to a test tube along with 2 mL of warmed distilled water at 37°C. The solution was observed for 8 minutes following incubation at 37°C for 4 minutes in the dark. Using a UV-visual spectrophotometer, absorbance was measured at 593 nm with methanol as the blank. A linear standard curve was generated using Trolox with concentrations of 100 and 1000 µM.

The findings were compared with Trolox, which was utilized as a positive control, and increased absorbance of the reaction mixture was analysed as an increase in the extract's reducing activity. The formula that was used to determine the sample's percent reduction in relation to the standard (Trolox) was as following:

Percentage of reduction power (%) = [1 − (1 – As/Ac)] x100

Where, AS= absorbance of sample and AC = absorbance of standard at maximum concentration tested.

Statistical Analysis

The results of TPC, DPPH and FRAP assays obtained from the UV-Vis spectrophotometer was further analysed in Microsoft Excel 2016. The analysed data was then subjected to one-way analysis of variance (ANOVA) in completely randomized designs to determine the interaction between the independent variables, and Post Hoc (Tukey's test) was used to compare mean values at confidence level 95% and α = 0.05. Differences were deemed significant when the p-value obtained was lower than 0.05. The data obtained were presented in mean value ± standard deviation of the mean. The relationship between the total phenolic content and the antioxidant activity was determined using Pearson's correlation coefficient (r) (Khaw *et al*., 2016). Statistical analysis was performed using Minitab 19 statistical software.

RESULT AND DISCUSSION

Determination of Total Phenolic Contents

Polyphenols, also known as phenols, are secondary plant metabolites that are found in all types of plants and plant-based products. Numerous phenolics have been found to have strong antioxidant activity levels (Razali *et al*., 2008; Rohman *et al*., 2010). Due to their redox characteristics, phenolic compounds primarily contribute to the overall antioxidant activities of plants. In general, phenolic compounds' actions for antioxidant activity involve scavenging lipid free radicals and halting the conversion of hydroperoxides into free radicals (Javanmardi *et al*., 2003; Li *et al*., 2009; Rohman *et al*., 2010). By employing gallic acid as a standard phenolic compound in the Folin-Ciocalteau (F-C) assay, the Total Phenolic Contents (TPC) of juice samples was calculated. The assay is a quick and straightforward procedure to characterize and standardize TPC of botanical materials. The F-C method involves oxidizing phenolics with a molybdotungstate in an F-C reagent to produce a coloured product with a maximum wavelength of 745–750nm (Prior *et al*., 2005; Rohman *et al*., 2010). Linear calibration curve of gallic acid with concentration range of 0.05-0.5 mg/ml and correlation coefficient (R2) = 0.9995 was obtained (Figure 1) and was used to determine TPC. TPC of all four juice samples; fresh and heat-treated juices, was determined and expressed in mg GAE/g. The result was then demonstrated as mean ± standard deviation as shown in Table 1.

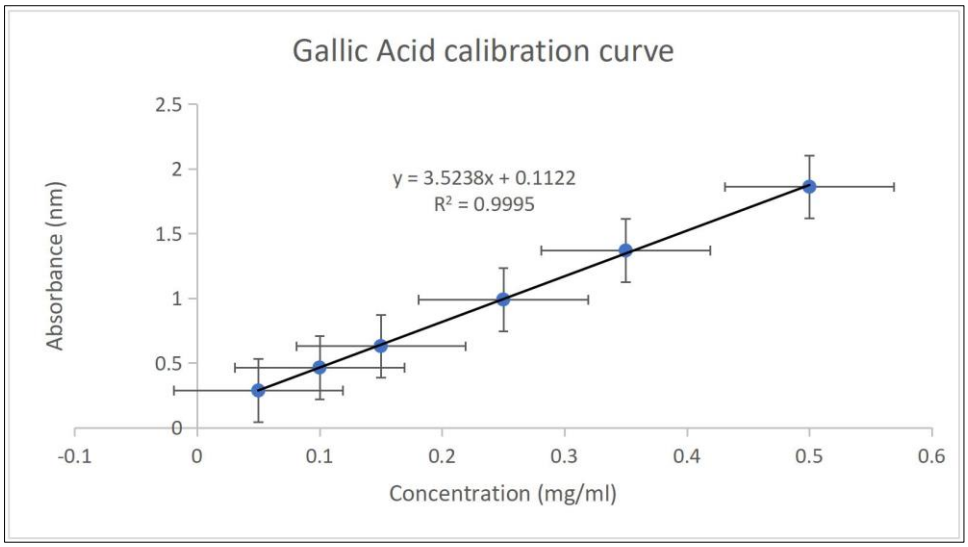

Figure 1: Callibration curve of gallic acid standard solutions

Table 1: Total phenolic contents of juice samples from raw and heat-treated Ambarella fruit

Preservation methods	Total phenolic contents (mg GAE/g)
No heat treatment	5.3002 ± 0.114a
Pasteurization	4.1473 ± 0.182b
Retort	4.2236 ± 0.214b
Water bath	3.6137 ± 0.174c

N = 4, replicates = 3
Means that do not share a letter are significantly different

Based on the data obtained, the range value obtained for the TPC for raw and heat-preserved Ambarella juices were 3.6137-5.3002 mg GAE/g. There was significant difference observed between the TPC values of all juice samples since the p-value obtained was ($p<0.05$). The highest TPC value among the juice preservation methods was detected in the raw juice (5.3002 ± 0.114 mg GAE/g), followed by retort (4.2236 ± 0.214 mg GAE/g), pasteurization (4.1473 ± 0.182 mg GAE/g), and the lowest TPC value was the water bath method (3.6137 ± 0.174 mg GAE/g). However, the values were considerably low compared to the one reported in previous study (Rahman *et al*., 2016) where the TPC values detected in Ambarella fruit extract was 27.08 ± 1.66 mg GAE/ g DW.

TPC values in fruit juices is influenced by a number of variables, including variation of the fruit types, storage conditions, and processing methods (Palety *et al*., 2020). Based on the study ((Islam *et al*., 2013; Rahman *et al*., 2016), there are a significant difference observed on the amount of total phenolic contents detected in the Ambarella variety in Bangladesh compared to those that grows in Malaysia, where the TPC value for Bangladesh variety was 659.74 ± 0.97 mg GAE/ g DW while Malaysia 27.08 ± 1.66 mg GAE/ g DW. This data supported the finding of another study (Shourove *et al*., 2020) which stated that cultivar variations, climate, geography of the environment, and the soil conditions also influenced the phenolic contents of the fruit.

The processing stages of fruit juice also gave a huge impact on the phenolic contents. In the previous study of (Rahman *et al*., 2016), a pure Ambarella extract from ground sample was used for determination of the total phenolic contents. In contrast, the Ambarella sample in this study was in the form of juice which underwent processing stages such as washing, peeling, blending and juicing. In comparison to scientific extraction procedure carried out in the previous study, blending step in the juice preparation stage might not be able to fully extract the phenolic components present in the fruit, which would cause the TPC values to deviate (Khaw *et al*., 2016).

Moreover, the differences between the TPC values obtained in this study and the previous study was dependent on the application of heat preservation method used. It can be observed that the TPC values of raw juice was higher than that of heat preserved juice. This can be explained by a finding from a study which stated that convectional thermal processing and organically cultivated food resulted in a severe reduction of phenolic content (Lima *et al*., 2009; Shourove *et al*., 2020). The heat treatment conditions strongly influenced the TPC contents that remains in the sample. Based on (Vikram *et al*., 2005; Shourove *et al*., 2020), pomegranate juice's total polyphenol content was reportedly impacted by heat treatment and it steadily dropped as temperature increased over time. Therefore, this explained why water bath method has the lowest TPC content since the juice was exposed to a high temperature (100ºC) for a longer

time (10 minutes) compared to pasteurization (85ºC at 30 seconds) and retort processing (121ºC in 1 minute). In essence, the clarifying, filtering, and pasteurization steps used to treat fruit juice will have a significant impact on TPC values because they may eliminate some of the phenolic compounds that were attached to the fibre and pectin in the fruit (Candrawinata *et al*., 2012; Palety *et al*., 2020).

Determination of Free Radical Scavenging Activity

The free radical scavenging activity of raw and heat-preserved Ambarella (Spondias dulcis) fruit juice was determined using DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. The maximal band of the DPPH radical's absorption ranges from 515 to 528 nm, making it a stable organic free radical. In this test, the antioxidants change the DPPH radical's (purple-colored) colour to diphenylpicryl-hydrazine, a yellow compound. The antioxidants' capacity to donate hydrogen determines how much colour changes. Ascorbic acid, tocopherol, cysteine, glutathione, gallic acid, and a few more substances have been discovered to be able reduce and decolorize DPPH radical.

The ascorbic acid standard curve with an R2 value of 0.988 and the percentage scavenging activity equation = (1 – As / Ac) x 100 was used to calculate the antioxidant content of each juice sample. The result was expressed in percentage (%) of free radical scavenging activity and the data was presented as mean ± standard deviation (Table 2).

Table 2: Percentage of scavenging activity of juice samples from raw and heat-treated Ambarella fruit

Preservation methods	Percentage of scavenging activity (%)
No heat treatment	53.83 ± 0.48b
Pasteurization	55.81 ± 0.55a
Water bath	43.57 ± 0.70c
Retort	33.44 ± 0.61d

N = 4, replicates = 3

Means that do not share a letter are significantly different

Referring to the data in Table 2, the range for the percentage of scavenging activity of raw and heat-preserved Ambarella fruit juices was 55.81-33.44%. A significant difference was observed between the percentage of scavenging activity of all juice samples since the p-value obtained was ($p<0.05$). The highest value of radical scavenging activities was observed in pasteurization method (55.81 ± 0.55%), followed by raw unpreserved juice (53.83 ± 0.48%), water bath method (43.57 ± 0.70%) and the lowest recorded was in retorted juice (33.44 ± 0.61%). Based on a literature (Rahman *et al*., 2016), the scavenging effect of Ambarella fruit (concentration of 5000 µg/ml) ranged from 76 to 83%. As compared to this present study, the values calculated for scavenging activities of Ambarella juices in this study were far lower.

This finding can be explained with relation to the implementation of heat treatment method to preserve the juice. Another study found that while antioxidant scavenging activity was best in fresh juice and diminished following thermal treatment, it can also remain unaltered under certain heat treatment conditions. However, antioxidant activity is destroyed after a 40-min heat treatment at 70 °C. The DPPH scavenging activity increased and then reduced again with increasing time during a 80°C (20 min) heat treatment (Ali *et al*., 2017; Shourove *et al*., 2020). In addition to that, the differences in value obtained between the raw Ambarella juice and that in (Rahman *et al*., 2016) might be due to other factors such as differences in method used for sample preparation and extrinsic conditions related to the sample.

According to (Rahman *et al*., 2016), the DPPH reduction was significantly associated with the extract's anti-oxidative and scavenging activity. The amount of DPPH reduction will depend on how many antioxidants are present in the extract. Greater scavenging potential was related to higher DPPH reduction; hence greater percentage of scavenging activity indicates that greater number of antioxidants present in the samples. To compare the result obtained, the presence of antioxidant compounds in all juice samples tested was in the order of pasteurized juice > raw juice > water bath juiced > retorted juice.

Contrary to the statement from (Rahman *et al.,* 2016) where fresh raw juice has the best scavenging effect, the result observed showed that pasteurized juice has the highest scavenging activity (55.81 ± 0.55%). One explanation for this outcome is because the use of external pressure encourages the breakdown of the bond between sugar and phenolic compounds, boosting the rate of phenolic compound extraction (Lopes *et al*., 2016; Hu *et al*., 2020). Another theory is that the disintegration of plant cells brought on by pasteurization facilitates the extraction of biologically active components (Wang *et al*., 2017; Hu *et al*., 2020). Since all the juice samples exhibited dose-dependent DPPH scavenging activity, these samples exhibit strong and considerable free radical scavenging activity. Conclusively, there is a significant correlation between phenolic content and both DPPH scavenging and antioxidant activities, indicating that phenolic compounds are likely in charge of the antioxidant activity (Rahman *et al*., 2016).

Determination of Reducing Power

In the Ferric Reducing Antioxidant Power (FRAP) assay, antioxidants (reductants) in the sample would cause Fe3+ to be reduced to Fe2+ by donating an electron away. By measuring the development of a colour complex at 700nm, it is possible to keep track of how much Fe2+ complex is produced during the reduction of Fe3+. An increase in absorbance suggests an increase in the reductive power. The potential antioxidant activity of a substance may be significantly indicated by the compound's reducing capacity. This would inhibit chain reactions that are started by free radicals by converting them into a more stable compound (Ardestani & Yazdanparast, 2007, Chung *et al*., 2006; Rekha *et al*., 2012). The data from UV-Vis spectrophotometer was analyzed, the calibration curve of Trolox with R2=0.9981 and reducing power equation = [1 − (1 – As / Ac)] x100 was used to determine the reducing power of each juice samples. The result was expressed in percentage (%) of reducing power and the data was presented as mean ± standard deviation (Table 3).

Table 3: Percentage of reducing power of juice samples from raw and heat-treated Ambarella fruit

Preservation methods	Percentage of reducing power (%)
No heat treatment	98.30 ± 0.85a
Pasteurization	76.20 ± 0.87b
Retort	69.67 ± 0.59c
Water bath	65.66 ± 0.68d

Means that do not share a letter are significantly different

According to the data obtained, the range for the percentage reducing power of the juice samples was 65.66-98.30%. ($P<0.05$) was observed from the ANOVA test indicating that there was a significant difference between the percentage of ferric reducing power of all juice samples. The highest ferric reducing power detected was in raw juice sample (98.30 ± 0.85%), followed by pasteurized juice (76.20 ± 0.87%) and retorted juice (69.67 ± 0.59%). The lowest reducing power recorded was in the water bathed juice sample (65.66 ± 0.68%). From the data obtained, it was observed that higher reducing power was detected in raw unpasteurized juice compared to heatpreserved juice samples.

Based on a study (Orak *et al*., 2012; Hu *et al*., 2020), a compound's reducing power was related to its ability to move electrons, which is also an indicator of antioxidant capacity. Raw juices had a high percentage reduction power since they contained more antioxidants than other types of juice samples. Commercially marketed fruit juices go through numerous industrial procedures such squeezing, pasteurization, and freezing that may influence antioxidant levels, according to (Densupsoontorn N *et al*., 2000; Palety *et al*., 2020). These variations in the antioxidant activity of fruit juices in the present research may be caused by the fruits chosen, the method utilized to extract the juice, the handling of the juice, the sample preparation and analysis methods used in the laboratory work (Sreekumar, S, *et al*., 2014; Palety *et al*., 2020).

Correlations between Total Phenolic Content and Antioxidant Activities

A correlation is simply a linear relationship between two variables, which means that if one variable increase or falls, the other variable also rises or falls. This relationship may be either positive—in which case both variables increase consistently—or negative—in which case one variable falls while the other increases. In this study, correlation coefficient was determined using Pearson's correlation coefficient test with 95% confidence interval. The result of total phenolic content's correlation with antioxidants activities (scavenging effect and reducing power) was expressed as (r) and then presented in Table 4.

Table 4: Correlation between TPC and antioxidant activities

	TPC	DPPH	FRAP
TPC	1.000	0.407	0.963
DPPH	0.407	1.000	0.615
FRAP	0.963	0.615	1.000

According to the table above, it can be observed that the total phenolic contents was positively correlated with both antioxidant assays DPPH (r = 0.407) and FRAP (r = 0.963). In addition, a positive and significant correlation between scavenging activity and reducing power was also observed (r = 0.615). This result demonstrated that phenolic compounds were the key source of antioxidant activity in terms of radical scavenging and ion reducing capacity (Khaw *et al.,* 2016). Though the r-value obtained between TPC and both assays were positive and significant, the correlation coefficient between TPC and DPPH was lower than that of FRAP. Based on a previous study (Rahman *et al*., 2016), in every evaluated system, extracts with the highest phenolic content should exhibit the greatest antioxidant capacity, whereas extracts with lower total phenolic contents should demonstrate weaker antioxidant activity.

The results obtained in this study however deviated the statement because the highest TPC values detected was in raw juice (5.3002 ± 0.114 mg GAE/g) but its' percentage scavenging activity was (53.83 ± 0.48%), which is second after pasteurized juice (55.81 ± 0.55%) with TPC value of (4.1473 ± 0.182 mg GAE/g). In contrast, the correlation coefficient between TPC and

FRAP was consistent with the study by (Rahman *et al*., 2016) since (r = 0.963). For example, raw juice with the highest phenolic content (5.3002 ± 0.114 mg GAE/g of dry weight) demonstrated high reducing activity (98.30 ± 0.85%), whereas water bath juice which had the lowest phenolic content (3.6137 ± 0.174 mg GAE/g of dry weight) demonstrated the lowest reducing effect (65.66 ± 0.68%).

Nevertheless, fruit juices' antioxidant contents, cannot totally be predicted based on their phenolic content only because the juices' vitamin C and carotenoids also take part in promoting antioxidant activities. (Almeida *et al*., 201; Khaw *et al*., 2016). Since other methanol-soluble chemicals, such methylxanthine or certain fruit pigments can also react with DPPH radicals, samples with low phenolic content may exhibit strong antioxidant activity (Belak *et al*., 2009; Khaw *et al*., 2016). Furthermore, by accounting for other non-phenolic reducing agents such organic acid, sugar, and ascorbic acid present throughout the Folin-Ciocalteu assay, the phenolic content may also be overestimated. Therefore, to provide more precise quantification of phenolic compounds in future studies, individual phenolic compound characterization via an accurate analytical platform is necessary (Khaw *et al*., 2016).

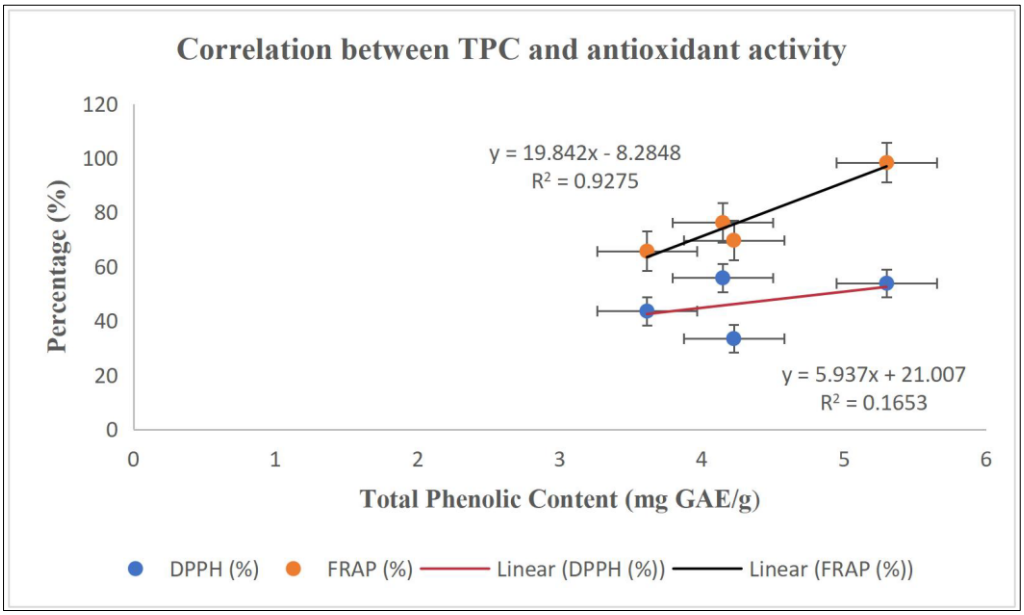

Figure 2: Correlation between total phenolic compounds and antioxidant assay

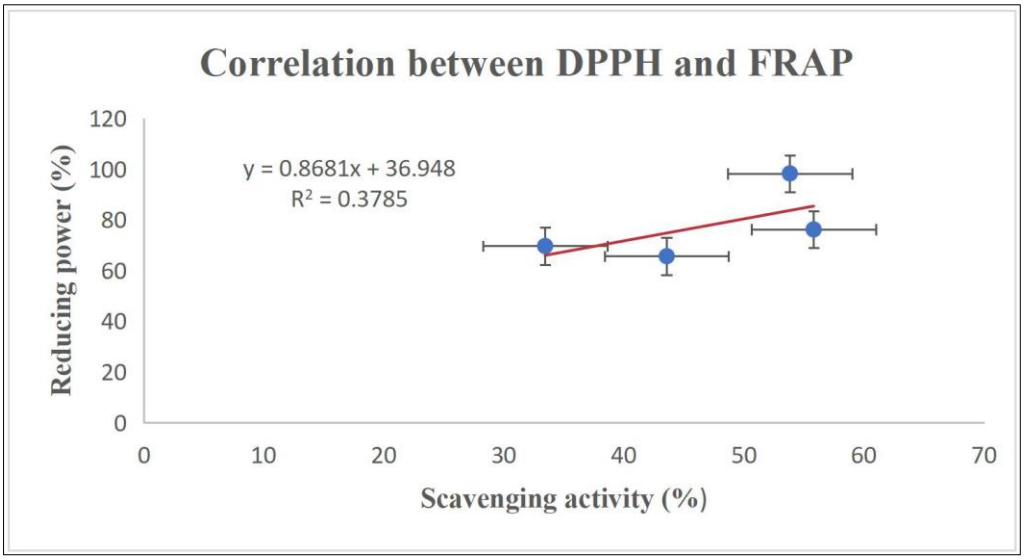

Figure 3: Correlation between scavenging activity and reducing power

CONCLUSION

In this study, Ambarella (Spondias dulcis) juice was treated with different preservation methods; pasteurization, water bath and retort processing. Antioxidant assays (Folin- Ciocalteu's assay, DPPH free radical scavenging activity and FRAP assay) was performed to compare and evaluate the best heat

preservation methods that will give lesser damage to the phytochemical compounds that exist naturally in the fruit. Overall, it can be concluded that total phenolic contents positively influenced the scavenging activity of DPPH (r = 0.407) and ferric reducing power (r = 0.963). From the result, it was observed that raw, unpasteurized juice sample retained the highest total phenolic contents as well as high percentage of both antioxidant activities (DPPH and FRAP). Juices that are sold in stores went through a variety of processing steps, which could cause nutrients lost. Therefore, it is usually advised to consume fresh fruit juice rather than fruit juice that has been processed commercially (Palety *et al*., 2020).

Though fresh juice is the best option to retain the natural phytochemical compounds in the fruits, not all people has the access or privileges of getting fresh stocks of fruits in their home. Hence why, from this study the option that was observed as the best to replace fresh juice was pasteurized juice. From the results obtained in this study, pasteurized juice showed comparatively high TPC value and higher antioxidant activity percentage compared to the water bath and retort method. While retaining a high level of antioxidants and also the flavour profile, pasteurization method also ensures that the drink is safe enough to consume. The functional components, sensory quality, and acceptability of tropical juices are generally unaffected by pasteurization (85 °C for 30 s followed by hot-filling) (Wurlitzer *et al*., 2019). Meanwhile, the other two methods might be less suitable to replace fresh juice since high temperature and long processing time resulted in more severe nutrients lost. In addition, it also affected the flavour profile and physical appearance of the juice. To sum up, to compensate for the inevitable nutrient loss in the juice during processing stages, the food industry can concentrate on creating novel functional meals and drinks with enhanced health advantages.

Acknowledgements

Gratitude to the Faculty of Science and Technology, Universiti Sains Islam Malaysia (USIM) for providing with the facilities and equipment's required throughout the research. A special thanks is also conveyed to the laboratory assistants who were involved either directly or indirectly in the journey to complete this research.

REFERENCES

- A Agbor, G., Vinson, J. A., & Donnelly, P. E. (2014). Folin-Ciocalteau Reagent for Polyphenolic Assay. *International Journal of Food Science, Nutrition and Dietetics*, (August), 147–156. https://doi.org/10.19070/2326-3350-1400028
- Aghajanzadeh, S., Ziaiifar, A. M., & Verkerk, R. (2021). Effect of thermal and nonthermal treatments on the color of citrus juice: A review. *Food Reviews International*, *00*(00), 1–23. https://doi.org/10.1080/87559129.2021.2012799
- Boyer, R., & McKinney, J. (2013). Boiling Water Bath Canning. *Virginia Cooperative Extension*, *1*(1), 1–15. Retrieved from https://pubs.ext.vt.edu/content/dam/pubs_ext_vt_edu/348/348-594/348594_pdf.pdf
- De Souza VR, Popovic V, Bissonnette S et al (2020) Quality changes in cold pressed juices after processing by high hydrostatic pressure, ultraviolet-c light and thermal treatment at commercial regimes. Innovative Food Science Emerging Technology 64:102398. https://doi.org/10.1016/j.ifset.2020.102398
- Dewan, F. (2020). Thermal Treatment of Food Preservation. *Bangabandhu Sheikh Mujibur Rahman Agricultural University*, (August), 1–15. https://doi.org/10.13140/RG.2.2.16633.49761/1
- Fiedor, J., & Burda, K. (2014). Potential role of carotenoids as antioxidants in human health and disease. *Nutrients*, *6*(2), 466–488. https://doi.org/10.3390/nu6020466 Gonçalves, A. C., Bento, C., Jesus, F., Alves, G., & Silva, L. R. (2018). Sweet Cherry phenolic compounds: Identification, characterization, and health benefits. *Studies in Natural Products Chemistry*, 31–78. https://doi.org/10.1016/b978-0-444-64179-3.00002-5
- Hu, Y. H., Wang, C. Y., & Chen, B. Y. (2020). Effects of high-pressure processing and thermal pasteurization on quality and microbiological safety of jaboticaba (Myrciaria cauliflora) juice during cold storage. *Journal of Food Science and Technology*, *57*(9), 3334–3344. https://doi.org/10.1007/s13197-020-04366-w
- Jana, H. (2016). Ambarella Tree : Considering Potentiality Needs More Focus in Indian agriculture. Rashtriya Krishi, 11(2).
- Jayarathna, P. L. I., Jayawardena, J. A. E. C., & Vanniarachchy, M. P. G. (2020). Identification of Physical, Chemical Properties and Flavor Profile of Spondias dulcis in Three Maturity Stages. *International Research Journal of Advanced Engineering and Science*, *5*(1), 208–211. https://doi.org/10.5281/zenodo.3695472
- Khaw, W. H, Haron, H & Chan, K. B (2016). Comparison of Total Phenolic Contents (TPC) and Antioxidant Activities of Fresh Fruit Juices, Commercial 100% Fruit Juices and Fruit Drinks. *Sains Malaysiana*. 45. 1319-1327.
- Koubala, B., Kansci, G., & Ralet, M. (2018). Ambarella— Spondias cytherea. In 2307758274 1575887181 S. Rodrigues, 2307758275 1575887181 S. E. Oliveira, & 2307758276 1575887181 B. E. De (Authors), *Exotic Fruits Reference Guide* (pp. 15-22). Amsterdam, Netherlands: Academic Press. doi: https://doi.org/10.1016/B978-0-12-803138-4.00005-8
- Lamuela-Raventós, R. M. (2017). Folin-CIOCALTEU method for the measurement of total

phenolic content and antioxidant capacity. Measurement of Antioxidant Activity & Capacity, 107–115. https://doi.org/10.1002/9781119135388.ch6

- Mahmood, T., &, Paramdeep Bagga, Mohammed Haris Siddiqui, S. F. (2011). Determination of total phenol & flavonoid content in seeds of Psoralea corylifolia. *An Indian Journal*, *7*(4), 171–173. Malaysian Food Regulation 1985, Part VIII (Fruit and fruit product), Regulation 235 (Fruit juice), 1985.
- Munteanu, I. G., & Apetrei, C. (2021). Analytical methods used in determining antioxidant activity: A review. *International Journal of Molecular Sciences*, 22(7). https://doi.org/10.3390/ijms22073380
- Oroian, M. and Escriche, I. (2015) Antioxidants: Characterization, Natural Sources, Extraction and Analysis. *Food Research International*, 74, 10-36. https://doi.org/10.1016/j.foodres.2015.04.018
- Palety, K. K., A.V, U., Narsaiah, T. B., Addepally, U., Sirisha, K., & A.S, H. (2020). Evaluation of Total Phenolics and Antioxidants of Fresh and Commercial Fruit Juices. *Journal of Scientific Research*, *64*(03), 50–53. https://doi.org/10.37398/jsr.2020.640310
- Pereira RN, Teixeira JA, Vicente AA *et al.,* (2018) Ohmic heating for the dairy industry: a potential technology to develop probiotic dairy foods in association with modifications of whey protein structure. Current Opinion in Food Science. 22:95–101. https://doi.org/10. 1016/j.cofs.2018.01.014
- Rahman, M. M., Khan, F. E., Das, R., & Hossain, M. A. (2016). Antioxidant activity and total phenolic content of some indigenous fruits of Bangladesh. *International Food Research Journal*, *23*(6), 2399–2404.
- Rajauria, G., & Tiwari, B. K. (2018). Fruit juices. *Fruit Juices,* 3-13. doi:10.1016/b978- 0-12-802230-6.00001-1
- Ramesh, M. N. (2020). Handbook of Food Preservation (3rd edition), Vol. Pasteurization and Food Preservation, pp.10-16. CRC Press. doi: https://doi.org/10.1201/9780429091483
- Rekha, c., poornima, g., manasa, m., abhipsa, v., devi, j. P., kumar, h. T. V., & kekuda, t. R. P. (2012). Ascorbic Acid, Total Phenol Content and Antioxidant Activity of Fresh Juices of Four Ripe and Unripe Citrus Fruits. Chemical Science Transactions, 1(2), 303–310. https://doi.org/10.7598/cst2012.182
- Renard, C. M. G. C., & Maingonnat, J. F. (2012). Thermal processing of fruits and fruit juices. *Thermal Food Processing: New Technologies and Quality Issues, Second Edition*, 413–438. https://doi.org/10.1201/b12112
- Rohman, A. Riyanto, S. Yuniarti, N. Saputra, W.R. Utami, R & Mulatsih. W (2010). Antioxidant activity, total phenolic, and total flavaonoid of extracts and fractions of red fruit (Pandanus conoideus Lam). *International Food Research Journal*, 106, 97–106. Sinan, K. I., Zengin, G., Zheleva-Dimitrova, D., Gevrenova, R., Picot-Allain, M. C. N., Dall'Acqua, S., … Mahomoodally, M. F. (2021). Exploring the chemical profiles and biological values of two spondias species (S. Dulcis and S. Mombin): Valuable sources of bioactive natural products. *Antioxidants*, *10*(11). https://doi.org/10.3390/antiox10111771
- Shourove, J. H., Zzaman, W., Chowdhury, R. S., & Hoque, M. M. (2020). Effect of Thermal Treatment on Physicochemical Stability and Antioxidant Properties of Locally Available Underutilized Star Fruit Juice. *Asian Food Science Journal*, (March), 41–53. https://doi.org/10.9734/afsj/2020/v14i330133
- Sridhar, A., Ponnuchamy, M., Kumar, P. S., & Kapoor, A. (2021). Food preservation techniques and nanotechnology for increased shelf life of fruits, vegetables, beverages and spices: a review. Environmental chemistry letters, 19(2), 1715–1735. https://doi.org/10.1007/s10311-020-01126-2
- Wang, Y., Li, W., Ma, Y., Zhao, X., & Zhang, C. (2018). Effect of Thermal Treatments on Quality and Aroma of Watermelon Juice. *Journal of Food Quality*, *2018*. https://doi.org/10.1155/2018/9242675
- Wurlitzer, N. J., Dionísio, A. P., Lima, J. R., Garruti, D. dos S., Silva Araújo, I. M. da, da Rocha, R. F. J., & Maia, J. L. (2019). Tropical fruit juice: effect of thermal treatment and storage time on sensory and functional properties. *Journal of Food Science and Technology*, 56(12), 5184–5193. https://doi.org/10.1007/s13197-019-03987-0
- Xiao, F., Xu, T., Lu, B., & Liu, R. (2020). Guidelines for antioxidant assays for food components. *Food Frontiers*, *1*(1), 60–69. https://doi.org/10.1002/fft2.10
- Young, A. J., & Lowe, G. L. (2018). Carotenoids—antioxidant properties. *Antioxidants*, *7*(2), 10–13. https://doi.org/10.3390/antiox7020028
- Zhong, Y., & Shahidi, F. (2015). Methods for the assessment of antioxidant activity in foods11This chapter is reproduced to a large extent from an article in press by the authors in the Journal of Functional Foods. *Handbook of Antioxidants for Food Preservation*, 287–333. https://doi.org/10.1016/b978-1-78242-089-7.00012-9.

Cite This Article: Hanis Nadia Y, I. N. Mohd Nasir, L Karim, Hafiza Y (2025). The Effects of Different Heat Preservation Methods on the Antioxidant Activity of Ambarella (Spondias Dulcis) Fruit Juice. *East African Scholars J Agri Life Sci, 8*(11), 312-321.